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FINAL REPORT

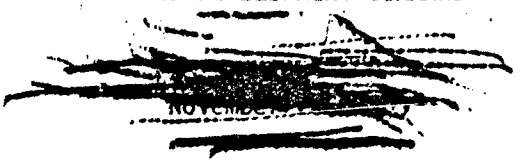
Multiple Animal Studies for Medical Chemical Defense
Program in Soldier/Patient Decontamination and Drug Development

on

TASK ORDER 84-4: TISSUE DISTRIBUTION OF ARSENIC IN THE RABBIT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF LEWISITE WITH OR WITHOUT BRITISH ANTI-LEWISITE THERAPY

to

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND



by

Dr. Ronald L. Joiner
Dr. H. Hugh Harroff, Jr.
Dr. Gerald L. Fisher
Mr. Thomas H. Snider
Mr. W. Bruce Keys, Jr.
Mrs. Robyn C. Kiser
Dr. Paul I. Feder

Contract No. DAMD17-83-C-3129

BATTELLE
Columbus Division
505 King Avenue
Columbus, Ohio 43201-2693

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U. S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 85-23, revised 1985).

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A task was performed to determine if multiple intramuscular injections of British Anti-Lewisite (BAL; or 2,3-dimercapto-1-propanol) administered to rabbits at a non-toxic dosage afforded therapeutic benefits following a challenge dose of Lewisite (L), with particular emphasis on determining if BAL mobilized arsenic (As) for accumulation in neural tissues.

BAL significantly reduced concentrations of As in blood, brain, spinal cord, lung, liver, testes, and kidneys. Arsenic accumulated in brain and spinal cord tissues in rabbits not receiving BAL therapy whereas, BAL therapy reduced As concentrations in these tissues to near the vehicle control level.

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Battelle
Columbus Division

Report

November 20, 1985

Ronald L. Joiner, Ph.D.
Study Director

H. Hugh Harrop, Jr., D.V.M.
Chief Veterinarian

FINAL REPORT

on

TISSUE DISTRIBUTION OF ARSENIC IN THE RABBIT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF LEWISITE WITH OR WITHOUT BRITISH ANTI-LEWISITE THERAPY

to

U. S. ARMY MEDICAL RESEARCH
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Ronald L. Joiner, Ph.D.
Study Director

H. Hugh Harroff, Jr., D.V.M.
Chief Veterinarian

Gerald L. Fisher, Ph.D.
Scientific Advisor

Thomas H. Snider, B.S.
Study Supervisor

Dr. Paul I. Feder, Ph.D.
Biostatistician

Ramona A. Mayer, B.A.
Quality Assurance
09 October 1985

EXECUTIVE SUMMARY

The objective of Task 84-4 was to determine if multiple intramuscular injections of British Anti-Lewisite (BAL; or 2, 3-dimercapto-1-propanol) administered to rabbits at a non-toxic dosage afforded therapeutic benefits following a challenge dose of Lewisite (L), with particular emphasis on determining if BAL mobilized arsenic (As) for accumulation in neural tissues.

Separate 14-day lethality dose-response curves were determined in rabbits for L administered subcutaneously (s.c.) on the dorsum and for BAL administered intramuscularly (i.m.) in the quadriceps. Challenge L dose levels of 2.4 mg/kg (~LD_{10}) and 3.5 mg/kg (~LD_{40}) were selected and a therapeutic dose level of 35 mg/kg was selected from the BAL non-toxic dose-response curve.

These dose levels were used in a dual-phase study to determine the efficacy of BAL in ameliorating the systemic toxicity of elemental As resulting from L exposure. Animals were dosed with L and subsequently either treated with BAL or not treated and sacrificed over a 4-day period. Tissue As distributions were determined by atomic absorption spectroscopy.

At both doses of L, BAL significantly reduced concentrations of As in blood, brain, spinal cord, lung, liver, testes, and kidneys. Arsenic accumulated in brain and spinal cord tissues in rabbits not receiving BAL therapy over the 4-day period, whereas BAL therapy reduced As concentrations in these tissues to near the vehicle control level. The results from this study suggest that As is mobilized but is not accumulated into neural tissues by BAL therapy.

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TISSUE DISTRIBUTION OF ARSENIC IN THE RABBIT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF LEWISITE WITH OR WITHOUT BRITISH ANTI-LEWISITE THERAPY

1.0 INTRODUCTION

Previous work by Hoover and Aposhian[1] suggested that the choice of British Anti-Lewisite (BAL; or 2,3-dimercapto-1-propanol) for treatment of arsenic (As) intoxication should be re-examined, based on brain As concentration data from 11 rabbits given 1 mg/kg of a solution of radiolabeled As acid ($^{74}As_5H_3O_4$) dissolved in an aqueous solution of sodium arsenite. Dithiol therapy was given at 1 hr after As dosing and consisted of either BAL or the sodium salt of 2,3-dimercapto-1-propane sulfonic acid (DMPS), given once i.m. at 200 µmol/kg. Animals (N = 3 for each therapy) were sacrificed 24 hr after As dosing. BAL therapy doubled the brain ^{74}As concentrations over normal saline controls, whereas DMPS reduced the ^{74}As levels to less than half that of the controls. In a separate study, 9 rabbits were given the same As challenge followed by either normal saline or BAL therapy, consisting of 4 i.m. treatments of 2.5 mg/kg (20 µmol/kg) each. As levels in brains collected 24 hr after As dosing were significantly elevated in the BAL group relative to controls.

The above results led to the work done at the Medical Research and Evaluation Facility (MREF) under Task 84-4. Task 84-4 was initiated in December 1984 under MREF Protocol 10 ("Subcutaneous Study for the Assessment of Lethality of Lewisite in the Rabbit") to determine a lethality dose-response curve for L administered s.c.. The task was continued under MREF Protocol 11 ("Assessment of Lethality of Multiple Intramuscular Doses of British Anti-Lewisite (BAL)") to determine a lethality dose-response curve for BAL administered i.m.

Dose levels of L and BAL were selected from the respective lethality dose-response curves for use in the two phases of MREF Protocol 12 ("Tissue Distribution of Arsenic in the Rabbit Following Administration of Lewisite With and Without BAL Therapy") performed in May and August 1985.

The objective of this Task was to determine As concentrations in selected tissues resulting from a challenge L dose followed by multiple administrations of BAL at a nontoxic dosage and to determine whether BAL mobilized As for accumulation in neural tissues of rabbits. In addition to brain and spinal cord, eight other tissues were selected for As analyses for comparison with data obtained by previous workers. Copies of the signed protocols are included as Appendix A.

2.0 MATERIALS AND METHODS

2.1 ANIMALS

Albino rabbits were chosen for this study on the basis of the extensive data base available for percutaneous application of toxic materials in this species. Equal numbers of 2.0- to 4.0-kg male New Zealand White (albino) rabbits from the Kings Wheel Rabbitry, 8085 Camp Road, Route 5, Mt. Vernon, Ohio 43050, were randomly assigned to treatment groups based on body weights so that body weight means and variance were homogeneous across groups. All animals were quarantined for at least 7 days at Battelle Columbus Laboratories' Animal Resources Facility at 505 King Avenue before being transported to MREF. Upon receipt at the Animal Resources Facility, the rabbits were ear tattooed for positive identification, weighed, sexed, and observed for signs of disease. At MREF, animals were acclimated for at least 24 hrs prior to being placed on study. At both facilities, housing was individual in stainless-steel, slotted cages equipped with automatic watering systems. Humidity was programmed and maintained at 50 percent ($\pm$10 percent) and temperature at 70 F ($\pm$5 F). Fluorescent lighting was maintained at a light/dark cycle of 12 hrs each per day. Purina Certified Rabbit Chow and water were available at all times during quarantine and holding. During the 24-hr test period, animals were given free access to water but were not given rabbit chow while in the treatment stanchions.

Battelle's Animal Resources Facilities have been registered with the U. S. Department of Agriculture (USDA) as a Research Facility (Number 31-21) since August 14, 1967, and are periodically inspected in accordance with the

provisions of the Federal Animal Welfare Act. In addition, animals for use in research are obtained only from laboratory animal suppliers duly licensed by the USDA. Battelle's statement of assurance regarding the Department of Health and Human Services policy on humane care of laboratory animals was accepted by the Office of Protection from Research Risks, National Institutes of Health on August 27, 1973. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 85-23), and/or in the regulations and standards as promulgated by the Agricultural Research Service, USDA, pursuant to the Laboratory Animal Welfare Act of August 24, 1966 as amended (P.L. 89-544 and P.L. 91-579).

On January 31, 1978, Battelle's Columbus Division received full accreditation of its animal care program and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. MREF is a part of the facilities granted full accreditation.

2.2 EXPERIMENTAL DESIGN

2.2.1 Lethality Studies

Separate acute toxicity studies (14-day LD_{50}) were performed in rabbits at doses bracketing the LD_{50}s estimated from the literature data for L administered s.c. (2.0 mg/kg) and for BAL administered i.m. into the femoral quadriceps (four injections of 24.8 mg/kg per injection). Both materials were dissolved in absolute ethanol for injection. Groups of eight male rabbits were randomly assigned according to weight to treatment groups for the 14-day studies. Sufficient numbers of groups were used with each treatment regimen to produce an LD_{50} (with at least five mortality fractions between 10 and 90 percent) and confidence limits. Duplicate 14-day LD_{50} determinations were performed for each material, and the results were pooled.

2.2.2 Mobilization Studies

Two groups of 50 animals each were dosed with L at the calculated LD_{10} (2.4 mg/kg) and LD_{40} (3.5 mg/kg) doses derived from the L lethality studies. BAL therapy was begun 1 hr later in half of the animals. BAL therapy consisted of four nontoxic injections (calculated LD_{01}, 35 mg/kg per injection) dissolved in ethanol and delivered at 4-hr intervals beginning 1 hr after the L dose. Dosing techniques were identical to those used in the acute toxicity studies.

Five animals were randomly selected and sacrificed by administration of T-61 euthanasia solution from each group at 4, 12, 24, 48, and 96 hr after the L dose. In addition, five ethanol-dosed control animals were sacrificed at 0 and at 96 hr. Blood, brain, spinal cord, liver, kidney, fat, testes, lung, L injection-site skin, and normal skin adjacent to L injection-site skin were sampled for histopathology and tissue As analysis. The treatment groups are defined below:

Group	Dose(mg/kg) L	Dose(mg/kg) BAL	Total Animals	Number of Animals Sacrificed for Tissue Sampling — Sacrifice Periods (hr) 0	4	12	24	48	96
I	2.4	35	50	0	5	5	5	5	5
II	2.4	0	50	0	5	5	5	5	5
III	0	0	10	5	-	-	-	-	5
IV	3.5	35	50	0	5	5	5	5	5
V	3.5	0	50	0	5	5	5	5	5
VI	0	0	10	5	-	-	-	-	5

2.3 EXPERIMENTAL COMPOUNDS

Goldshield ethanol (absolute) was obtained from U. S. Industrial Chemicals Co. (Newark, NJ). L was supplied by U. S. Army Medical Research and Development Command (USAMRDC). Undiluted BAL (2,3-dimercapto-1-propanol) was

obtained from either Aldrich Chemical Company (Milwaukee, WI) or Hynson, Westcott & Dunning (Baltimore, MD). L and BAL were supplied with the following information:

	L	BAL
Purity (%)	95.8	95.0
Density (g/ml)	1.88	1.239
Known impurities	4.0% Dichloro (2-chlorovinyl) arsine, cis-isomer	Max. 2% 1,2,3-trimercapto-propane
Color	Light amber	Clear,colorless
Appearance	Slightly oily liquid	Viscous, oily liquid

Battelle did not confirm the purity, density, identities of impurities, or other information supplied by USAMRDC or the commercial vendor. Dose analyses were not performed since at the time of the study a specific definitive method for L was not available at MREF.

2.4 PREPARATION OF ANIMALS

Prior to injection, each animal was weighed and randomly assigned by body weight to a test group so that body weight means and variance were homogeneous across groups. For treatment with either L or the vehicle, animals were clipped of hair at the dorsum using an Oster animal clipper with a No. 40 blade. They were anesthetized by i.m. injection in the gluteal region with a mixture of Ketamine (35 mg/kg) and xylazine (5 mg/kg). The Ketamine dose of 35 mg/kg, twice that called for in MREF Protocol 12, was necessary due to the deeper-than-usual plane of anesthesia needed for s.c. administration of L. The unconscious animals were then placed in stainless-steel stanchions and transported to a toxic fume hood for dosing.

For treatment with BAL, hair was clipped bilaterally at the femoral quadriceps, and two dosing sites approximately 2 cm apart were marked on the skin with a felt-tipped pen over each femoral quadricep for BAL dosing sites (4 sites altogether).

2.5 APPLICATION OF TEST MATERIALS

For treatment with L, a single dose (LD_{10} or LD_{40}) at a constant volume of 33.3 µl of L diluted in ethanol was administered using a 250- or 500-µl Hamilton gas-tight syringe fitted with a 23-gauge disposable needle. The dose was administered by lifting the skin from the musculature at the midline of the back, inserting the needle, rotating it 90 degrees, and depositing the dose s.c. Light pressure was applied with a butyl rubber-gloved fingertip at the injection site during withdrawal of the needle to reduce seepage.

For treatment with BAL, the animals were dosed without prior anesthesia at each of the four marked sites with 4-hr intervals between doses. Each injection was administered with a 500-µl Hamilton gas-tight syringe fitted with a 23-gauge needle at a dosage of 66.7 µl/kg of BAL diluted in ethanol. The BAL doses were deposited in or near the femoral quadriceps, alternating hind limbs with each dose. Dosing was performed in front of a hood to minimize potential personnel exposure to BAL vapor.

2.6 DECONTAMINATION PROCEDURES

Immediately after dosing, the L injection site was decontaminated with a pad soaked in 5 percent sodium hypochlorite solution, rinsed twice with distilled water, and blotted dry with a plastic-backed paper towel. The animals remained in the dosing hood in stanchions for 10 min after dosing. The dose site was then decontaminated and rinsed as before, and the animals were transferred to holding cages, where they stayed for the remainder of the study.

2.7 MORTALITY EVALUATIONS

Animals were inspected periodically for signs of toxicity over the remainder of the dosing day and twice daily over the remainder of the 14-day period. Mortality was recorded on the morning of the day following dosing and

at subsequent 24-hr intervals. Euthanasia was performed on all surviving animals using T-61® at the end of each 14-day test period. No tissues were collected from rabbits used in the 14-day lethality studies.

The mortality data from the initial studies of L alone and BAL alone were used to construct 14-day lethality dose-response curves for each material. Data from replicate LD_{50} studies were pooled into composite lethality dose-response curves for L and separately for BAL. The LD_{10} and LD_{40} were selected from the L composite curve, and the LD_{01} was selected from the BAL composite curve for use in the tissue As distribution portion of this Task.

2.8 NECROPSY AND TISSUE COLLECTION

The order of animals used in the As distribution studies was randomized to ensure that there was no bias due to body weight during the entire dosing period. Animals not surviving to scheduled sacrifice were discarded from the study and replaced with the next available animal in the dosing sequence (randomized prior to study start). Actual time of sacrifice was usually within 1 hr of the scheduled time of sacrifice.

Samples of blood (5 ml), injection-site skin, normal skin adjacent to the injection site, spinal cord, abdominal fat, brain, liver, kidneys, testes, and lungs were collected and weighed (except blood). Portions of each (except blood) were sampled and preserved in 10 percent neutral buffered formalin for histopathology if deemed necessary. Injection-site skin in L-dosed animals was defined as the area of the dorsum skin around the injection site that exhibited reddening and thickening and yellow, caseous material s.c. The injection site was typically circumscribed on the under surface by a yellow band. The brain was bisected sagittally. For brain, lungs, and testes, the left specimen was collected for possible histopathology and the right specimen was used for determining As concentration. The left kidney was bisected longitudinally and the right kidney was bisected transversely. One-half of each kidney was collected for histopathology, and the other half was stored at -20 C for determining As concentration.

2.9 TISSUE ARSENIC DETERMINATIONS

The specific procedure for As analysis is detailed in the attached revised protocol (Appendix A) and support documentation is given in Appendix B. In general, tissue samples were thawed and those weighing more than 1 g were homogenized. Skin samples were homogenized to a liquid consistency with 10 ml of As-free water (less than 0.5 ng As/ml). An approximate 1-g aliquot was taken from the homogenized sample and weighed on an analytical balance. Samples of tissues weighing 1 g or less (e.g., testis) were used *in toto* without homogenization.

Samples were digested by adding a solution of concentrated nitric and sulfuric acids and magnesium nitrate and by heating the mixture to fuming. Hydrogen peroxide solution was added and heated in steps until solutions were clear. Sample solutions were dried on a hot plate and reconstituted with an acidic solution. A mercury hydride generation system was used to form arsine gas by sodium borohydride reduction of sample As; the As gas was quantified with an atomic absorption spectrophotometer.

The wide range of tissue As concentrations required that various amounts of reconstituted sample be subjected to the reduction step to quantify the As present within the detection range of the spectrophotometer. Thus, lower detection limits were affected by the concentrations of As and varied from sample to sample.

2.10 STATISTICAL ANALYSES

Statistical tests were conducted for each replicate lethality study and for the ability to pool the replicates for a composite LD_{50}. Mean tissue As levels were calculated and an analysis of variance (ANOVA) and a regression analysis was done for each tissue.

2.10.1 Lethality Studies

The 14-day lethality studies were conducted in a stepwise fashion. Doses were adjusted in subsequent replicate studies based on results obtained previously. A completed replicate was defined as containing at least five

dose groups having between 10 percent and 90 percent mortality. LD_{50} estimates, associated confidence intervals, and slopes were calculated separately for each replicate based on the 2-parameter $\log_{10}$ probit model (Finney, D. J., Probit Analysis, Third Ed. 1971).

Data from each 14-day study were examined for their approximation to the theoretical sigmoidal dose-response curve and were accepted or rejected based on the chi-square (X^2) value and degrees of freedom (df). Background lethality was not incorporated into the model since the studies were 14-day tests in otherwise healthy rabbits, and no background lethality was expected.

Each set of L and BAL data was examined for poolability into a composite of the replicates. X^2 values and df from probit analyses were summed across the replicate LD_{50} values. Delta X^2 was calculated as the difference between the composite X^2 and the sum of the replicate X^2 values. Delta df was calculated as the difference between the composite df and the sum of the replicate dfs. The delta X^2 was then compared with the critical X^2, with delta df at alpha = 0.05, from a table of X^2. If delta X^2 was less than critical X^2, then the null hypothesis (H_0:no replicate effect) was accepted, and the data were pooled. However, if delta X^2 was greater than critical X^2, then the null hypothesis (H_0:no replicate effect) was rejected, and the data were not pooled. In this case, an outlier replicate would be discarded and delta X^2 recalculated or another replicate LD_{50} determined and the procedure repeated. Doses for the final portion of the task involving L with and without BAL therapy were derived from the respective composite lethality dose-response curves.

2.10.2 Tissue Arsenic Distribution Studies

2.10.2.1 Outlier Screens

Although we were careful during tissue sampling and weighing to avoid cross-contamination among tissues, the possibility of accidental transfer of As via gloves and instruments, particularly via the homogenizer, remained a concern. Thus, data from the tissue As distribution studies were screened for outliers. The variables screened included whole organ weights

(brain, liver, kidneys, testes, and lungs) and $\log_{10}$ transformed tissue As concentrations (blood, brain, spinal cord, right lung, liver, right testis, kidney, abdominal fat, dose-site skin, and normal skin).

A conservative decision level of plus or minus three standard deviations (alpha = 0.0026, two-sided) from the sample mean was used. Each sample (n = 60) consisted of residuals formed by the differences between observed values and mean values predicted by the second-order polynomial regression curves over all sacrifice periods. The two-sided method of Grubbs[2], used at alpha = 0.0026, was incorporated into a SAS (Statistical Analysis System, Inc., Cary, NC) algorithm that input the data as a univariate sample and calculated studentized residuals in a single-parameter regression model. The program then identified and eliminated the most extreme outlier (if any) in either tail. The procedure repeated itself until no outliers remained.

2.10.2.2 Analytic Approaches to the Data

Mean As concentrations were determined for every tissue sampled at each sacrifice interval. The very low levels of As in some samples of tissue prevented a definitive assay by atomic absorption. Results were then expressed as less than the methodologic detection limit calculated for that particular sample, which was based on its As concentration and the volume sampled for analysis.

The effect of BAL therapy on As concentration was determined as a function of time after dosing with L (with repeated administrations of BAL therapy). More specifically, the methods used in this analysis were designed to determine:

- Differences among mean As concentrations in various tissues of animals receiving L and BAL, receiving L only, or receiving only a vehicle control
- Sensitivity of an ANOVA approach versus a regression approach
- The effect of actual and expected (nominal) time of sacrifice on statistical analysis

- The effect of ignoring the detection limit values (i.e., defining each calculated limit as the assay value) on the statistical analysis. This was a concern in spite of the relatively low incidence of analyses below detection limits.

2.10.2.3 Analysis of Variance Evaluations

The basic ANOVA approach was conducted using a one-way model. Each treatment in the analysis represented a unique combination of experimental treatment and nominal time on test. Thus, animals receiving L and BAL or L only produced a total of 10 treatments, while the vehicle controls produced two treatments. At each nominal time point (4, 12, 24, 48, and 96 hr), differences between the estimated means of the As concentration (as $\log_{10}$) of animals treated with L and BAL and animals receiving L only were calculated. The $\log_{10}$ transformation was used to equalize variation across time. The standard errors of these differences and a t statistic for the differences were also calculated. Poolability tests were conducted between the vehicle controls at 0 and 96 hr. Finally, contrasts were made between the average of the vehicle controls and L with BAL or L only treated animals at each time point.

The basic ANOVA approach was modified to include a continuous covariate to reflect the difference between the actual time of sample collection (time on test) and the nominal time of sample collection. The same contrasts were made based on adjusted means, using the ANOVA with the time covariate, as were made using the basic ANOVA.

Each of the above analyses was run twice, using different values for As concentrations determined below the detection limit in each run. In one case, values less than the detection limit were set to zero, and in the other case, they were set to the actual detection limits. This test was to determine whether setting unknown assay levels to the upper or lower extreme made any difference in the analyses; i.e., whether the precision of the

analytical method at its lower end was critical to the conclusions reached. Thus, for the ANOVA approach, four separate runs were conducted:

- No covariate, As levels < detection limit = 0
- No covariate, As levels < detection limit = detection limit
- Covariate, As levels < detection limit = 0
- Covariate, As levels < detection limit = detection limit.

2.10.2.4 Regression Evaluations

A preliminary inspection of the data revealed smooth, monotonic time trends that appeared to be adequately modeled by a quadratic regression. A $\log_{10}$ transformation of the As concentrations and organ and body weights was performed to homogenize variance across sacrifice times.

The regression analysis chosen fit a second-order polynomial model to the time trends of the $\log_{10}$ As concentration. Dummy 0-1 variables were used to estimate separate slopes and intercepts for L with BAL and L only treatments, as well as to estimate the means of the vehicle controls pooled over time. The same contrasts made with the ANOVA approaches were made in this analysis. All regression model contrasts were made between predicted means using estimates of variance determined by the model at the specified times. Two runs were made, with As levels less than detection limit values set either to zero or to the detection limits.

2.10.2.5 Comparison of ANOVA and Regression Evaluations

The six separate statistical analyses were compared for the two most important responses in the study, brain and blood As concentrations. Brain was chosen because it is a primary target organ for As. Blood was chosen because it is a good index of the systemic As content. For these two responses, there was little difference either among the four ANOVA models or between the two regression models in analysis results. Since the results were similar, selection of an optimal model was somewhat arbitrary. For lack of better criteria, we chose the variance and normality of residuals respective

to each probability plot of the residuals. Among the ANOVA models, the one with a time covariate and with As levels less than detection limits set to the detection limits had the smallest residual variance and rendered the most normally distributed residuals. Between the two regression models, the one with As levels less than detection limits set to the detection limits also had the smallest variance and rendered more normally distributed residuals.

A power test was then applied between these two models to determine which gave the overall greater sensitivity to detect effects of BAL therapy. The test showed that the regression model had equivalent sensitivity to the ANOVA model at 0 and 96 hr, the ends of the regression curve. However, between the ends of the curve, the regression model was 1.3 to 1.7 times more powerful in detecting test effects than the ANOVA model. Thus, we applied to all tissue As concentration data the regression model with As concentrations less than calculated detection limits set equal to detection limits.

2.10.2.6 Whole Organ Arsenic Content

The regression model was applied to whole organ As content calculated as the product of whole organ weight (for paired organs, both members) and As concentration for that tissue. Whole organ As content for brain, liver, kidneys, lungs, and testes was calculated and analyzed for the effect of BAL therapy. A $\log_{10}$ transformation was performed prior to analysis to equalize variance across time. The whole organ As content variables were not directly subjected to the outlier screen since they were products of variables already screened.

2.10.2.7 Whole Organ Arsenic Content Expressed as a Portion of Total Dose

Total As dose applied (T, in mg) was calculated for each animal that received L as

$$T = 0.3613\ BW \cdot D$$

where

0.3613 was the fraction of As in L,
BW was the animal body weight (kg) at the study start, and
D was the L dosage level in mg/kg.

The whole organ As content for brain, lungs, liver, kidneys, and testes expressed as a portion of the total As dose was calculated by dividing the whole organ As content by T. The regression model was applied to each of the resulting percent variables. A $\log_{10}$ transformation was performed prior to analysis to equalize variance across time. These variables were not directly subjected to the outlier screen since they were derived from variables already screened.

3.0 RESULTS

Tables are presented in Appendix C and Figures are presented in Appendix D.

3.1 ACUTE TOXICITY STUDIES

The results of the acute toxicity tests for range-finding and definite 14-day LD_{50} studies for both L and BAL are presented in the following sections.

3.1.1 Lewisite Range-finding Studies

Five groups of four animals per group were used in a 9-day range-finding study. Dosages for this study, based on log intervals of 0.2 around the estimated[3] subcutaneous LD_{50} of 2.0 mg/kg, were 0.8, 1.3, 2.0, 3.2, and

5.0 mg/kg. The end point of this study was three doses that produced mortalities between 0 and 100 percent, with all deaths occurring within the first 6 days of the 9-day observation period. The dosages and corresponding mortality profiles are presented in Table 3.1.1.

3.1.2 Lewisite 14-day LD_{50} Studies

The dosages and corresponding mortality profile with time for each of the LD_{50} replicates for L are given in Table 3.1.2. Most deaths occurred in the first 7 days after dosing, but some were scattered out even to day 14. A probit plot of these data, excluding 0 and 100 percent lethalities, is presented in Figure 3.1.1. The LD_{50} for the first replicate, which consisted of 2 days of testing, was 3.61 mg/kg, with a lower confidence limit of 3.21 and an upper limit of 4.13. The slope for the curve was 7.05. The second replicate had an LD_{50} of 4.13 mg/kg, with lower and upper limits of 3.47 and 6.00, respectively; the slope was 5.45.

Tests of poolability showed the two replicates to be consistent and poolable ($P > 0.05$). The composite LD_{50}, based on the pooled data from both replicates, was 3.79 mg/kg, with a lower limit of 3.44 and an upper limit of 4.25. The slope for the composite LD_{50} was 6.39, plus or minus 2.17 (two standard errors). A summary of the probit analyses is presented in Table 3.1.5.

The calculated LD_{10} and LD_{40} were 2.4 mg/kg and 3.5 mg/kg, respectively. These dosages were selected for the As distribution portion of this Task to provide an effect dose (LD_{10}) with many survivors and one close to the LD_{50} but on the conservative side (LD_{40}) to ensure that sufficient animals would finish the study. Probit analysis results that were considered in the selection of L doses are presented in Table 3.1.6.

3.1.3 BAL Range-finding Studies

Seven groups (including one ethanol control) of two animals per group were used in each of two replicate 8-day BAL range-finding studies. Doses for these were based on $\log_{10}$ increments of 0.15 around the estimated[4] LD_{50} of 24.8 mg/kg given four times (total accumulation LD_{50} of

99.2 mg/kg). The end point for these studies was two doses that produced mortalities between 0 and 100 percent. All deaths occurred within the first 5 days of the 8-day observation period. The dosages and corresponding mortality profiles with time are presented in Table 3.1.3.

3.1.4 BAL 14-day LD_{50} Studies

The dosages and corresponding mortality profile with time for each of the LD_{50} replicates for BAL are given in Table 3.1.4. A probit plot of these data, excluding 0 and 100 percent lethalities, is presented in Figure 3.1.2. The LD_{50} for the first replicate, which consisted of 2 days of dosing, was 52.5 mg/kg, with a lower confidence limit of 49.2 and an upper limit of 56.3. The slope for the curve was 16.0. The second replicate had an LD_{50} of 51.8 mg/kg, with lower and upper limits of 45.7 and 55.1, respectively; the slope was 14.9.

Tests of poolability showed the two replicates to be consistent and poolable ($P > 0.05$). The composite LD_{50}, based on the pooled data from both replicates, was 52.2 mg/kg, with a lower limit of 49.8 and an upper limit of 54.5. The slope for the composite LD_{50} was 15.8, plus or minus 5.4 (two standard errors). The composite LD_{01} was 37.2 mg/kg, with lower and upper confidence limits of 30.8 and 41.0. We chose 35.0 mg/kg for the tissue arsenic distribution portion of this task because this dose produced no lethality in the LD_{50} studies. Data summaries of the acute toxicity studies are presented in Table 3.1.5. A summary of the L and BAL doses used in the tissue As distribution studies is presented in Table 3.1.6.

3.2 TISSUE ARSENIC DISTRIBUTION STUDIES

Results of two studies to determine As distribution in rabbit tissues following L administration at either 2.4 or 3.5 mg/kg with or without BAL therapy are presented separately in the following sections.

3.2.1 Results of Dosing L at the LD_{10} (2.4 mg/kg) With and Without BAL Therapy

3.2.1.1 Whole Organ Weights

Whole organ weights for brain, lungs, liver, kidneys, testes, and dose-site skin are presented by treatment group and by sacrifice time in Tables 3.2.1 through 3.2.6, respectively. Dose-site skin weights for the vehicle control group are not presented, since lesions were not well defined at the dose site in these animals.

Results of outlier tests on organ weight variables are indicated on the respective tables. An outlier brain weight for animal number B1358 is indicated by an asterisk in Table 3.2.1. All other organ weight data were retained by the outlier screen and are summarized in Table 3.2.7, which presents the group mean and standard deviation at each time period. Vehicle control data for animals nominally sacrificed immediately after ethanol injection are presented at 4 hr after dosing to facilitate visual comparisons among the groups. Statistical equivalence ($P > 0.01$, two-sided) between two group means or among all three group means is indicated by a bracket. Statistically significant ($P < 0.01$) differences are implied by the absence of a bracket for all other comparisons (i.e., L alone versus L and BAL, L alone versus vehicle controls, and L and BAL versus vehicle controls).

Regression analyses of absolute (not $\log_{10}$-transformed) organ weight data revealed no statistically significant differences among group means at any sacrifice period for brain, kidneys, and testes weights.

There was no statistically significant effect of BAL therapy on mean lung weight except at 24 hr after L dosing, which was due to the presence of one unusually large lung (37.74 g) in an animal (B1421) of the group that received no BAL therapy. This finding was not considered treatment related. At 4 hr, the mean lung weight for the group without BAL therapy was statistically different from the vehicle control group mean, but not from the mean of the group receiving BAL therapy. At 12 and 48 hr, the BAL therapy group mean and the vehicle control group mean were significantly different, but there was no difference between therapy and no-therapy group means. By 96 hr after dosing, the lung weight means from all three groups were equivalent.

Liver weight means were equivalent across treatment groups through 48 hr after dosing. A steady decrease in liver weight for the group that received no BAL therapy resulted in a statistically significant decrement relative to the other groups at 96 hr.

Dose-site skin weights were analyzed for only the groups that received L, since the vehicle control animals did not exhibit a well-defined lesion at the dose site. Dose-site skin weights were equivalent irrespective of therapy at 4 and 96 hr after dosing. However, at 12, 24, and 48 hr, the mean dose-site skin weight for the no-therapy group was significantly greater than that for the BAL-therapy group. These data suggest that BAL therapy significantly reduced dermal swelling at the interim times.

3.2.1.2 Tissue Arsenic Distribution - Concentration Variables

Arsenic concentrations for whole blood, brain, spinal cord, right lung, liver, right testis, kidney, abdominal fat, dose-site skin, and normal skin adjacent to the dose site are presented by treatment group and by nominal sacrifice time in Tables 3.2.8 through 3.2.17 respectively. The tabular data are plotted with mean regression curves in Figures 3.2.1 through 3.2.10 respectively.

Two outlier brain As levels are indicated by asterisks in Table 3.2.9. All other tissue As data were retained by the outlier screens and are summarized in Table 3.2.18, which presents the group mean and standard deviation at each time period. Statistical equivalence is indicated by a bracket. Regression analysis was performed on the $\log_{10}$-transformed tissue As data. The $\log_{10}$ transformation was necessary to equalize variance across sacrifice time periods.

Mean blood As levels at 4 hr after L dosing were the same (approximately 470 ng/g) for both groups of L-dosed animals, irrespective of therapy. Blood As levels decreased in both groups through 96 hr, but the decrease was significantly accelerated by BAL therapy, especially in the first 24 hr after dosing. The effect associated with BAL therapy was a significant decrease in mean blood As at 12, 24, 48, and 96 hr after dosing. At 96 hr,

mean blood As in the no-therapy group (90 ng/g) was approximately twice that in the BAL-therapy group (41 ng/g), and both were significantly greater than that for vehicle controls (24 ng/g).

Mean brain As levels at 4 hr were equivalent (approximately 170 ng/g) in L-dosed animals, irrespective of BAL therapy. Mean brain As levels in the group that received no therapy increased to 206 ng/g at 96 hr, whereas in the group that received BAL therapy, mean brain As decreased to 25 ng/g at 96 hr. The difference between the curves was significant ($P < 0.01$) at every sacrifice period after 4 hr. The means of brain As levels in both L-dosed groups at 96 hr were significantly greater than the mean for vehicle controls.

Mean spinal cord As levels were initially significantly greater in BAL-treated animals than in their no-therapy counterparts. However, spinal cord As levels increased in animals not receiving BAL therapy and rapidly decreased in animals receiving BAL therapy (to 118 and 21 ng/g, respectively) at 96 hr. The decrease due to BAL therapy was significant at 24, 48, and 96 hr after dosing. Both group means at 96 hr were significantly greater than controls.

Arsenic concentrations in both groups decreased with time for lung, liver, kidney, fat, dose-site skin, and normal skin. BAL therapy significantly ($P < 0.01$) enhanced the elimination of arsenic from lung, liver, and kidney at all time periods after 4 hr. Arsenic levels in fat, dose-site skin, and normal skin were numerically (but not statistically) higher at 4 and 12 hr with BAL therapy than without it. Therapeutic effects of BAL were not statistically evident in abdominal fat As concentrations at any time period.

In general, mean As levels from all tissues of L-dosed animals were significantly elevated at all time periods relative to the vehicle-only controls. Exceptions to this were seen in testis and in fat, for which mean As in the BAL group decreased to levels statistically indistinguishable from controls at 96 hr.

3.2.1.3 Tissue Arsenic Distribution - Whole Organ Content Variables

Whole organ As content data for brain, lungs, liver, kidneys, testes, and dose-site skin are presented by treatment groups and by sacrifice time in Tables 3.2.19 through 3.2.24 respectively. The tabular data are plotted with mean regression curves in Figures 3.2.11 through 3.2.16 respectively. The whole organ variables were not directly subjected to the outlier screen since they were products of variables already screened for outliers. A $\log_{10}$ transformation was applied to the whole organ As content data prior to statistical analysis to equalize variance across time. The whole organ As content data are summarized in Table 3.2.25, which presents the group mean and standard deviation at each time period. Statistical equivalence is indicated by a bracket.

Mean whole organ As contents for brain, lungs, liver, kidneys, and dose-site skin were initially (i.e., at 4 hr after dosing) statistically equivalent in the two L-dosed groups, irrespective of BAL therapy. In testes, the total As content was initially significantly higher with BAL than without it. Total As in brain increased in the no-therapy group but was significantly lower in the BAL-therapy group at 12, 24, 48, and 96 hr. In all other organs analyzed, total As content decreased after 4 hr in both groups but was significantly accelerated by BAL therapy. BAL therapy was significant in aiding the elimination of As from lungs, liver, and kidneys at 12, 24, 48, and 96 hr. The effect of BAL therapy was not significant for total As content in testes and dose-site skin at 12 and 96 hr.

In general, all whole organ mean As content levels of L-dosed animals were significantly greater than means for controls at all times. Exceptions to this were observed in brain, lungs, and kidneys, for which BAL therapy reduced As content to near the control level at 96 hr, and in testes at 24, 48, and 96 hr.

3.2.1.4 Tissue Arsenic Distribution - Whole Organ Content Expressed as a Percent of Total Dose

Whole organ As content for brain, lungs, liver, kidneys, testes, and dose-site skin expressed as a percent of the total As dose for each animal that received L is presented by treatment group and sacrifice time in Tables

3.2.26 through 3.2.31. These variables were calculated to reduce variability due to animal size and to facilitate comparisons with data of previous studies. A $\log_{10}$ transformation was applied to the percent whole organ As content data prior to statistical analysis to equalize variance across time. The percent whole organ As content data are summarized in Table 3.2.32, which presents the group mean and standard deviation at each time period. Statistical equivalence is indicated by a bracket.

The effect of BAL therapy was significant at the same times for these variables as previously presented for absolute whole organ As content in brain, kidneys, and dose-site skin. However, in lungs and liver, the initial (4-hr) percent As content was significantly lower in the BAL-therapy group, and in lungs the final (96-hr) levels were equivalent. In addition, BAL therapy was significantly beneficial in testes at 48 hr only. These data were not plotted due to similarity of results to the absolute whole organ As content variables.

3.2.2 Results of Dosing L at the LD_{40} (3.5 mg/kg) With and Without BAL Therapy

3.2.2.1 Whole Organ Weights

Whole organ weights for brain, lungs, liver, kidneys, testes, and dose-site skin are presented by treatment group and by sacrifice time in Tables 3.2.33 through 3.2.38 respectively. Dose-site skin weights for the vehicle control group are not presented, since lesions were not well defined at the dose site in these animals.

All organ weight data were retained by the outlier screen and are summarized in Table 3.2.39, which presents the group mean and standard deviation at each time period. Statistical equivalence ($P > 0.01$, two-sided) between two group means or among all three group means is indicated by a bracket. Statistically significant ($P < 0.01$) differences are implied by the absence of a bracket for all other comparisons (i.e., L alone versus L and BAL, L alone versus vehicle controls, and L and BAL versus vehicle controls). An alpha decision level of 0.01 was used to determine statistical significance.

Regression analyses of $\log_{10}$-transformed organ weight data revealed no statistically significant differences among group means at any sacrifice period for weights of brain, lungs, liver, and testes. For kidney weights, there were no significant differences among the groups at 4, 12, and 24 hr after dosing. At 48 and 96 hr, mean kidneys weight for the no-therapy group was significantly greater than that for both the BAL-therapy group and the vehicle controls (which were statistically indistinguishable).

Dose-site skin weights were analyzed for only the groups that received L, since the vehicle control animals did not exhibit a well-defined lesion at the dose site. Dose-site skin weights were equivalent irrespective of therapy at 4 and 96 hr after dosing. However, at 12, 24, and 48 hr, the mean dose-site skin weight for the no-therapy group was significantly greater than that for the BAL-therapy group. These data suggest that BAL therapy partially but significantly prevented dermal swelling at the interim times.

3.2.2.2 Tissue Arsenic Distribution - Concentration Variables

As concentrations for whole blood, brain, spinal cord, right lung, liver, kidney, right testis, abdominal fat, dose-site skin, and normal skin adjacent to the dose site are presented by treatment group and by nominal sacrifice time in Tables 3.2.40 through 3.2.49 respectively. The tabular data are plotted with regression curves in Figures 3.2.17 through 3.2.26 respectively.

An outlier kidney As concentration for animal number B4963 is indicated by an asterisk in Table 3.2.45. All other tissue As data were retained by the outlier screens and are summarized in Table 3.2.50, which presents the group mean and standard deviation at each time period. Statistical equivalence between two or among three groups is indicated by a bracket. Regression analysis was performed on the $\log_{10}$-transformed tissue As data.

Mean whole blood As levels at 4 hr after L dosing was approximately 440 ng/g for both L-dosed groups, irrespective of BAL therapy. Blood As levels decreased in both groups through 96 hr, but the decrease was significantly accelerated by BAL therapy, especially in the first 24 hr after dosing. The effect associated with BAL therapy was a significant decrement in

mean blood As levels at 12, 24, 48, and 96 hr. At 96 hr, mean blood As in the no-therapy group (103 ng/g) was almost five times that in the BAL-therapy group (22 ng/g), and both were significantly greater than that for vehicle controls (7 ng/g).

Mean brain As levels at 4 hr were equivalent (approximately 200 ng/g) in L-dosed animals, irrespective of BAL therapy. From the 4-hr level, mean brain As in the no-therapy group increased to 309 ng/g at 96 hr, whereas in the BAL-therapy group, mean brain As decreased to 37 ng/g at 96 hr. The difference between the curves was significant ($P < 0.01$) at every sacrifice period after 4 hr. At 96 hr, brain As means for both L-dosed groups were statistically greater than that for the vehicle controls.

Mean spinal cord As in the BAL-therapy group (390 ng/g) was initially (4 hr) significantly greater than that in the no-therapy group (127 ng/g). However, at 12 hr after dosing and thereafter, mean spinal cord As was greater in the no-therapy group. The effect associated with BAL therapy was a significant decrement in As at 12, 24, 48, and 96 hr. At 96 hr, the no-therapy group spinal cord mean As level was 274 ng/g, the BAL-therapy group mean was 33 ng/g, and both were significantly greater than the vehicle control mean (17 ng/g).

Mean As levels in the non-neural tissues generally decreased with time for both L-dosed groups. Arsenic concentrations in right lung and liver were significantly lower in the BAL-treated group than in the no-therapy group at all sacrifice times. Arsenic concentrations in right testis and kidney samples were equivalent (irrespective of BAL therapy at 4 hr), but were significantly lower in the BAL-therapy group than in the no-therapy group at 12, 24, 48, and 96 hr. Liver and right testis As levels in the no-therapy group increased from hr 4 to 12 and from hr 4 to 24, respectively, and decreased thereafter.

Fat As levels were significantly greater in the BAL-therapy group (2034 ng/g) than in the no-therapy group (326 ng/g) at 4 hr. However, by 48 and 96 hr, BAL therapy had reduced As levels to significantly less than those of the no-therapy group. The 96-hr BAL-therapy group mean fat As level was statistically indistinguishable from the vehicle control mean. There was generally no significant effect of BAL therapy on dose-site and normal skin As levels. The initial mean normal skin As level of 300 ng/g remained practically unchanged throughout the study.

Except as mentioned above for fat at 96 hr, all tissue As means were significantly greater in both L-dosed groups than in the vehicle controls at all time periods.

3.2.2.3 Tissue Arsenic Distribution - Whole Organ Content Variables

Total As content data for brain, lungs, liver, kidneys, testes, and dose-site skin are presented by treatment group and by sacrifice time in Tables 3.2.51 through 3.2.56 respectively. The tabular data are plotted with mean regression curves in Figures 3.2.27 through 3.2.32 respectively. The data are summarized in Table 3.2.57, which presents the group mean and standard deviation at each time period. Statistical equivalence between two groups or among all three groups is indicated by a bracket.

Mean total As content for brain, kidneys, and testes were statistically equivalent at 4 hr after dosing in the two L-dosed groups, irrespective of BAL therapy. Thereafter, total brain and testes As levels generally increased for the no-therapy group and generally decreased for the BAL-therapy group. Total As levels in kidneys decreased in both L-dosed groups. The difference associated with BAL therapy in brain, kidneys, and testes was significant ($P < 0.01$) at 12, 24, 48, and 96 hr after dosing.

Total liver As levels in the no-therapy group increased from hr 4 to 12 and decreased thereafter. Total liver As levels in the BAL-therapy group were decreased from the 4-hr level at all later time periods. BAL therapy produced a significant reduction in liver As content at all time periods. Total lung As decreased from the 4-hr levels in both groups, and BAL therapy produced a significant decrement in lung As content at all time periods. The effect of BAL therapy was not significant for total As content in dose-site skin at any time periods.

In general, mean total As contents for the five organs analyzed (and excluding dose-site skin) were statistically greater in both L-dosed groups at all times than in the vehicle controls. Exceptions were observed in testes, where total As contents were reduced by BAL therapy at 24, 48, and 96 hr to levels statistically indistinguishable from the vehicle controls.

3.2.2.4 Tissue Arsenic Distribution - Whole Organ Content Expressed as a Percent of Total Dose

Whole organ As content for brain, lungs, liver, kidneys, testes, and dose-site skin expressed as a percent of the total As dose for each animal that received L is presented by treatment group and sacrifice time in Tables 3.2.58 through 3.2.63 respectively. These variables were calculated to reduce variability due to animal size and to facilitate comparisons with data of previous studies. A $\log_{10}$ transformation was applied to the percent whole organ As content data prior to statistical analysis to equalize variance across time. The percent whole organ As content data are summarized in Table 3.2.64, which presents the group mean and standard deviation at each time period. Statistical equivalence is indicated by a bracket.

The effect of BAL therapy was significant at the same times for these variables as previously presented for absolute whole organ As content in brain, lungs, liver, testes, and dose-site skin. However, in kidneys the initial (4-hr) and final (96-hr) levels were equivalent between treatment groups. These data were not plotted due to similarity of results to the absolute whole organ As content variables.

3.2.3 Comparisons of Results from Tissue Arsenic Distribution Studies

3.2.3.1 Tissue Arsenic Concentrations

Regression curves from both phases of the tissue As distribution studies are plotted for As concentrations in whole blood, brain, spinal cord, right lung, liver, kidney, right testis, abdominal fat, dose-site skin, and normal skin in Figures 3.2.33 through 3.2.42 respectively. Vehicle control data from both phases of the studies were combined to form the vehicle control curve.

Blood As levels for all L-dosed groups were approximately 450 ng/g at 4 hr, irrespective of L dose and BAL therapy. Blood As curves for the no-therapy groups were almost identical and were at higher levels than either of the BAL-therapy groups at times later than 4 hr after dosing. The 96-hr blood As levels for both L-dosed groups with BAL therapy were approximately the same.

Brain As levels for all four L-dosed groups were approximately 170 ng/g at 4 hr, irrespective of L dose level and BAL therapy. BAL therapy caused brain As levels to decrease at nearly identical rates for the first 12 hr after dosing, and 96-hr brain As levels were approximately the same, irrespective of L dose level. Without BAL therapy, As accumulation in brain was linear from a 2.4 mg/kg dose of L and increased to a plateau from a 3.5 mg/kg dose of L. The final concentrations reflected the difference in initial doses; i.e., the final concentration from the 3.5 mg/kg dose group (309 ng/g) was 50 percent greater than that from the 2.4 mg/kg dose group (206 ng/g).

Spinal cord As levels in BAL-therapy groups were initially more than twice the levels of the no-therapy groups at 4 hr. Thereafter, BAL therapy aided in the elimination of As, irrespective of the L dose level, to reduce As levels to near the vehicle control level by 96 hr. In the no-therapy groups, As from a 3.5 mg/kg dose accumulated (the mean predicted by the regression model was approximately 240 ng/g) to almost twice the level observed from a 2.4 mg/kg dose (the predicted mean was approximately 125 ng/g).

Lung As levels dropped with time for all L-dosed groups. In both the BAL-therapy groups and the no-therapy groups, lung As levels were greater in the 3.5 mg/kg L dose group than in the 2.4 mg/kg L dose group. The same pattern was also observed for kidney As concentrations.

Liver and testis As accumulated for up to 24 hr after dosing in the 3.5 mg/kg L dose, no-therapy group before decreasing. Final (96-hr) liver and testis As levels in the BAL-therapy groups were near normal levels.

Fat As levels were remarkably higher (2,034 ng/g) in the 3.5 mg/kg L dose, BAL-therapy groups than in the others at 4 hr. It decreased rapidly to near control levels at 96 hr. Fat As for the 3.5 mg/kg L dose, no-therapy counterpart group remained elevated through 96 hr.

Dose-site skin As levels appeared unaffected by BAL therapy at both L dose levels. Final As levels in the 3.5 mg/kg groups were approximately twice those in the 2.4 mg/kg groups. Normal skin As levels in the 3.5 mg/kg L dose groups were also approximately twice those in the 2.4 mg/kg groups at all time periods. At both dose levels, normal skin As levels decreased rapidly with BAL therapy for the first 24 hr and slowly increased from 48 to 96 hr.

3.2.3.2 Whole Organ Arsenic Content

Regression curves from both phases of the tissue As distribution studies are plotted for whole brain, lungs, liver, kidneys, testes, and dose-site skin in Figures 3.2.43 through 3.2.48 respectively. Vehicle control data from both phases of the studies were combined to form the vehicle control curve.

The whole organ As content mirrored the data presented for tissue As concentrations for all tissues except testes and dose-site skin. Total As content in testes from the no-therapy group at 3.5 mg/kg L dose increased during the first 24 hr after dosing and decreased slightly to 0.58 µg at hr 96. At the 2.4 mg/kg L dose with no therapy, the total testes As was relatively stable between approximately 0.20 µg and 0.25 µg for the duration of the experiment.

Total As content in dose-site skin was higher in the no-therapy groups at both dosages than in the corresponding BAL-therapy groups after 4 hr. Since dose-site skin As concentrations were nearly identical irrespective of therapy at each dosage (see Figure 3.2.41), the separation between total As content curves for a given dosage (Figure 3.2.48) also indicates the degree of effect of BAL therapy on injection-site skin lesion weights. That is, the separation between the no-therapy and BAL-therapy curves at 12, 24, and 48 hr in Figure 3.2.48 reiterates the results of the dose-site skin weight analyses summarized in Tables 3.2.7 and 3.2.39. The two no-therapy curves were nearly parallel, and the two BAL-therapy curves were nearly parallel. This suggests that in either case of L/no therapy or L/BAL therapy, the rate of As clearance from the dose site was constant over the range of dosages administered. This may mean that at the 2.4 mg/kg L dosage, As was in sufficient excess relative to BAL, so that an increase of L to 3.5 mg/kg did not increase the rate of As elimination from the injection-site skin.

4.0 DISCUSSION

Separate LD_{50} estimates were determined in lethality studies in rabbits for L dosed s.c. and for BAL dosed i.m. in two replicates. Results from the replicates in each study were poolable, and the composite LD_{50} was calculated by pooling the data from both replicates.

The 14-day LD_{50} for L, derived using 136 rabbits, was 3.79 mg/kg. This was almost twice the dosage (2 mg/kg) reported by the U. S. Army[3] on which range-finding study doses were based. The Army LD_{50} figure was not accompanied by experimental details as to the number of rabbits used, whether a vehicle solvent was used, or the duration of observations for lethality. The 95 percent confidence limits for the LD_{10} and LD_{40} for L reported here were less than 20 percent removed from the estimated levels of 2.4 and 3.5 mg/kg, respectively. Based on the reproducibility of our data (implicit in the poolability tests conducted) and the breadth of the 95 percent confidence limits, we used our composite probit analysis in determining the LD_{10} and LD_{40} of L for the tissue distribution studies.

The 14-day LD_{50} for BAL, derived using 144 rabbits, was 52.2 mg/kg per injection in a regimen of four injections for a total dose of 208.8 mg/kg. This was more than twice the LD_{50} of 99 mg/kg reported in the literature[4] for rabbits given BAL i.m. as Dimercaprol Injection, USP (70:20:10, peanut oil:benzyl benzoate:BAL w/w solution). In the present studies, BAL was administered without oil or stabilizer in an ethanol solution. Based on the reproducibility of our data and the 95 percent confidence limits of the LD_{01} in the composite probit analysis for BAL (less than 20 percent removed), we used our estimated LD_{01} as an approximate optimal dose (i.e., high enough to be therapeutic yet nonlethal) in the tissue distribution studies.

A quantitative analytical method was developed to determine As concentration in rabbit tissues. The method included tissue homogenization (except blood), acid digestion, and reconstitution to prepare samples for hydride generation and As determination via flameless atomic absorption spectrophotometry. The limit of As detection by this method was 5 ng/g (5 ppb), with recovery averaging 90 percent for organic As and 114 percent for inorganic As spiked in rabbit blood samples.

Arsenic concentrations in all tissues were significantly higher in all L-dosed animals at all time periods when compared to controls, except for testes and fat As levels which were similar to control values at 96 hr. Arsenic concentrations in both BAL-treated and untreated animals at both dose levels decreased with time in blood, lungs, liver, kidneys, fat, and skin (dosed and adjacent). BAL therapy significantly enhanced the elimination of As from lung, liver, and kidney tissues at both dose levels from 12 hr to the end of the study at 96 hr.

Blood As levels were similar at 4 hr after dosing in both L-dosed groups, irrespective of BAL therapy. The BAL therapy speeded the elimination of As from the blood at both dose levels. The final 96-hr As concentrations in blood were significantly greater in the no-therapy groups at both dose levels than in BAL-treated groups and vehicle controls.

Brain As levels were similar in all L-treated groups at 4 hr after dosing, irrespective of dose or therapy. BAL therapy significantly reduced brain As levels from that time period to the end of the study at both L dose levels, whereas As concentrations in brain tissue from no-therapy groups at both dose levels increased with time.

Aposhian and coworkers[1,6] found that BAL given i.m. to rabbits 1 hr after s.c. injection of radiolabeled arsenic acid dissolved in an aqueous solution of unlabeled sodium arsenite significantly increased the ^{74}As content of the brain 24 hr after As administration. Aposhian reported similar results for multiple doses of BAL given from 1 to 13 hr following As dosing. The differences in the two sets of data may be due to the different chemical forms and valence states of arsenicals used, i.e., Aposhian used arsenic acid (valence state +5) and we used an organic arsenical (valence state +3).

The results of our study are consistent with other published data on tissue distribution and elimination patterns in rats[7-10] and in rabbits[10-12]. Marafante and coworkers[11,12] reported that inorganic As was poorly retained in rabbit tissues over a 144-hr period, with the liver, lungs, kidneys, and spleen having the largest initial concentrations at 5 hr after dosing. All tissue concentrations decreased from 5 hr to the end of the 144-hr study. Graziano et al.[7] showed similar data for rat tissues following inorganic As administration via food and BAL administration, with As

concentrations in liver, kidneys, spleen, and brain of BAL-treated rats significantly lower than in untreated rats. In particular, BAL treatment significantly reduced brain As concentrations five-fold over no treatment.

In conclusion, the data from our study support the effectiveness of BAL therapy in cases of L exposure, particularly in reducing the As concentration in target tissues (brain, spinal cord). Our data do not show As accumulation in brain tissue of rabbits given L followed by BAL therapy, and are consistent with published reports by other authors who analyzed As concentrations in rabbit and rat tissues.

Additional studies are needed to compare organic (L) with inorganic (sodium arsenite) arsenicals against BAL, DMSA, and/or DMPS in the rabbit or other laboratory animal models to support the data collected in this study. A reduced study design could be used to minimize time, animal usage, and cost constraints, but the design should permit concomitant comparison of two species with two chelating materials against both forms of arsenic.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of the study are contained in Battelle Laboratory Record Book Nos. MREF-28, MREF-33, MREF-36, and MREF-51. All prestudy animal quarantine and observation records are on file at MREF. All original data, as well as the original final report, will be maintained at MREF until forwarded to USAMRDC at the conclusion of the project or until microfiched and permanently archived at Battelle.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest degree of the principal contributors in this study are presented in the following list:

Name	Title	Degree
Dr. Ronald L. Joiner	Study Director	Ph.D.
Dr. H. Hugh Harroff, Jr.	Chief Veterinarian	D.V.M.
Dr. Gerald L. Fisher	Scientific Advisor	Ph.D.
Thomas H. Snider	Study Supervisor	B.S.
Robyn C. Kiser	Technical Supervisor	B.S.
W. Bruce Keys	Technical Supervisor	M.B.A.
Timothy Hayes	Analytical Chemist	B.S.
Dr. Paul I. Feder	Biostatistician	Ph.D.
Ramona A. Mayer	Quality Assurance	B.A.

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APPENDIX A

MREF Protocol 10 --- "Subcutaneous Study for the Assessment of Lethality of Lewisite in the Rabbit"

MREF Protocol 11 --- "Assessment of Lethality of Multiple Intramuscular Doses of British Anti-Lewisite (BAL)"

MREF Protocol 12 --- "Tissue Distribution of Arsenic in the Rabbit Following Administration of Lewisite With and Without BAL Therapy"

Subcutaneous Study for the Assessment of Lethality of Lewisite in the Rabbit

Study performed by Battelle Columbus Laboratories
505 King Avenue, Columbus, Ohio 43201

1. Study Director: Ronald L. Joiner, Ph.D.

2. Veterinarian: H. Hugh Harroff, Jr., D.V.M.

3. Sponsor: U.S. Army Medical Research and Development Command

4. Sponsor Monitor: LTC Howard Johnson, USAMRICD

5. Objective:

To determine the LD_{50} of Lewisite when subcutaneously administered to the rabbit. A preliminary LD_{50} range-finding study is conducted to select the dose levels for the lethality study in the rabbit.

6. Experimental Design:

A. Test System

Albino rabbits were chosen for this study on the basis on the extensive data base available for this species.

(1) Animals -- New Zealand White (albino) male rabbits, supplied by Kings Wheel Rabbitry, Mt. Vernon, Ohio.

(2) Initial Weight -- 2.0 to 4.0 kilograms.

(3) Quarantine -- Rabbits are held in isolation and observed for clinical illness for at least 7 days prior to transport to West Jefferson for study initiation.

(4) Acclimation -- All animals are held at the Medical Research and Evaluation Facility for at least 24 hours prior to study initiation.

(5) Selection -- Animals selected after the minimum 7-day quarantine period are in good physical condition based on appearance. Rabbits are weighed and assigned to groups based on body weight.

(6) Animal Identification -- All animals are ear tattooed to retain positive identification during animal handling and observations. Cage cards are color-coded by group.

(7) Housing -- Animals are housed individually in stainless steel, slotted metabolic cages equipped with automatic watering systems.

(8) Lighting -- Fluorescent lighting, light/dark cycle is 12 hours each per day.

(9) Temperature -- Maintained at 70F (±5F).

(10) Humidity -- Maintained at 50% (±10%).

(11) Diet -- Purina Certified Rabbit Chow pellets are available at all times. No contaminants are known to be present in the feed which would interfere or affect the results of the study.

(12) Water Supply -- Water is supplied from the public water system and given ad libitum. No contaminants are known to be present in the water which would affect the results of the study.

B. Test Material

(1) Lewisite (dichloro-2-chlorovinylarsine) is supplied by the USAMRDC/ICD. Purity, appropriate identification (batch number, lot number, state), and stability data are supplied by the USAMRDC/ICD. Purity and stability are confirmed periodically by Battelle for materiel stored at the Hazardous Materials Laboratory.

(2) Surety, security, and safety procedures for the use of Lewisite are thoroughly outlined in facility plans, in personnel requirements for qualifications to work with agents, and in agent storage and use standard operating procedures. Specific procedures have been included in this protocol to ensure the safety of the personnel conducting this experiment.

C. Test Groups

The determination of the lethality of Lewisite in rabbits following subcutaneous administration is divided into three distinct phases. Phase 1 is a range-finding effort to determine the doses for the Phase 2 study to determine the LD_{50} of Lewisite. Phase 3 is a replication of the LD_{50}, adjusting doses as necessary.

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(1) Range-Finding Study -- The acute 14-day LD_{50} range-finding study of subcutaneously administered Lewisite is performed in 6 groups of rabbits (2 males/group) at doses bracketting the estimated LD_{50} (2.0 mg/kg) at 0.2 log increments. The test article is suspended in polyethylene glycol 200 (PEG 200) or other suitable solvent and administered by subcutaneous injection to the dorsal surface (back) in a region mid-way between the shoulders and the rump. An additional group of 2 male rabbits is similarly administered only the vehicle as shown below.

Group	Number of Male Rabbits	Dosage(mg/kg)
1	2	0 (vehicle only)
2	2	0.50
3	2	0.80
4	2	1.26
5	2	2.0
6	2	3.17
7	2	5.02

(2) Lethality Study -- The acute 14-day LD_{50} study of subcutaneously administered Lewisite is performed in at least 5 groups (but not more than 8 groups) of rabbits (8 males/group) at doses bracketting the estimated LD_{50} determined in the preliminary range-finding study. The test article is suspended in PEG 200 and administered as for the range-finding study. An additional group of 8 male rabbits is similarly administered the vehicle as shown below.

Group	Number of Male Rabbits	Dosage (mg/kg)
1	8	0 (vehicle only)
2	8	*
3	8	*
4	8	*
5	8	*
6	8	*
7 (if needed)	8	*
8 (if needed)	8	*
9 (if needed)	8	*

(*) Exact dosage levels are based on results of the previous range-finding study. The test article is administered by

subcutaneous injection. A sufficient number of groups are used to determine an appropriate LD_{50} with confidence limits.

All groups are treated during the same day to minimize daily experimental variation.

(3) Replication of Lethality Study -- The lethality study is repeated, adjusting doses as necessary to produce a valid LD_{50} with acceptable confidence intervals.

D. Study Preparation

(1) Animals -- One day prior to the start of the study, the back of each animal is clipped free of hair from the shoulders to the rump using a small animal clipper. This is done to visually assure appropriate dosage administration and to facilitate decontamina tion of the injection site.

(2) Anesthesia -- Rabbits are given anesthetic doses of a Rompun/Ketamine mixture by intramuscular injection.

(3) Marking Test Sites -- Rabbits are placed in a metal restraining box to restrict movement. An area for injection, about one square centimeter, is then marked on the back of each animal with a water-based ink.

E. Application of Agent

(1) Lewisite is injected using a glass syringe with a reusable platinum needle or with disposable stainless steel needles, which are immediately placed in decontaminating solution after use.

(2) The subcutaneous injections are administered by first lifting the skin from the musculature and then piercing the skin with the syringe needle.

(3) Each animal receives a single bolus injection of the test article or vehicle. The time of administration is recorded for each animal.

(4) All dosages are administered while the animals are in an approved chemical fume hood.

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F. Decontamination

(1) Following dose administration, the area of injection is decontaminated with 5% sodium hypochlorite by wiping the area with a pad drenched with the decontaminant. The injection site is then blotted dry with absorbent plastic-backed toweling. (2)The injection site of all animals is inspected after the last rabbit has been dosed. Animals are kept in the restrainers in the fume hood for two hours after dosing. After that time they are returned to the stainless steel metabolic holding cages where they are housed individually for the remainder of the study. In the event ulceration of the injection site occurs, animal collars will be used to prevent rabbits from disturbing the region of inflammation. Supportive treatment will be administered if it does not interfere with experimental results. Severely ulcerated animals will be terminated as moribund.

G. Specific Procedures

(1) Exposure and decontamination timing is controlled by one investigator who also maintains the laboratory notebook. A second investigator prepares the decontaminating materials and delivers them to the operating investigator in proper sequence and timing. The third operating investigator administers injections and performs decontaminating procedures while wearing butyl gloves and a butyl apron. A fourth investigator maintains a supply of rabbits from the preparation area to the exposure hoods and reports signs of toxicity or death of exposed rabbits to the reporting investigator.

(2) All animals are inspected after test article administration, the test site is wiped with 5% sodium hypochlorite to remove possible residual material, and the animals maintained in the fume hood for two hours. Animals are then transfered to holding cages for the remainder of the study.

(3) Observations are made for signs of toxicity at least once every hour after dosing for the remainder of the work day. Mortality is recorded on the morning of the day following exposure. The condition of survivors is also recorded. Daily individual observations, with morning and afternoon checks for physical signs of toxicity, are recorded for the remainder of the study. When possible, the onset and duration of signs are ascertained and described.

Revised October 10, 1984

(4) All surviving animals are euthanized 14 days after dosage administration by an intravenous overdose injection of T-61.

7. Necropsy and Histopathology:

Gross post-mortem examinations will not be performed for any animals during the study. No tissues will be saved for histopathology and all carcasses will be discarded.

8. Statistical Methods:

An LD_{50} calculation, slope, and 95 % confidence interval are made based on the results of the 24-hour and 14-day survival data. The calculation is performed according to the procedure of Finney, Probit Analyses, 3rd Ed. (1971), or by other suitable techniques.

9. Records to be Maintained:

A. Compound inventory, specifications, and usage

B. Dosage preparation and administration

C. Animal data

D. Clinical observations

E. Mortality

F. Proof of decontamination and disposal records

10. Reports:

A final report will be prepared and submitted within 30 days after completion of the task. It includes the following:

1. Signature page for key study individuals and their responsibilities
2. Experimental design
3. Animal supplier
4. Test animal selection criteria
5. Test material description and preparation
6. Treatment procedures
7. Description of clinical observations

Revised October 10, 1984

11. Study Approval:

A. For Battelle:

Ronald L. Joiner, Ph.D.
Study Director

October 16, 1984
Date

H. Hugh Harroff, Jr. D.V.M.
H. Hugh Harroff, Jr., D.V.M.
Chief Veterinarian

October 16, 1984
Date

B. For USAMRDC:

LTC Howard Johnson, D.V.M.
Sponsor Monitor

30 Oct 84
Date

Revised October 10, 1984

Assessment of Lethality of Multiple Intramuscular Doses of British Anti-Lewisite (BAL)

Study performed by Battelle Columbus Laboratories
505 King Avenue, Columbus, Ohio 43201

1. Study Director: Ronald L. Joiner, Ph.D.

2. Veterinarian: H. Hugh Harroff, Jr., D.V.M.

3. Sponsor: U.S. Army Medical Research and Development Command

4. Sponsor Monitor: LTC Howard Johnson, USAMRICD

5. Objective:

To determine the LD_{50} of British Anti-Lewisite when administered by intramuscular injection in the rabbit. The dose levels administered will be selected from the results of a preliminary LD_{50} range-finding study in this species.

6. Experimental Design:

A. Test System

Albino rabbits were chosen for this study on the basis on the extensive data base available for this species.

(1) Animals -- New Zealand White (albino) male rabbits, supplied by Kings Wheel Rabbitry, Mt. Vernon, Ohio.

(2) Initial Weight -- 2.0 to 4.0 kilograms.

(3) Quarantine -- Rabbits are held in isolation and observed for clinical illness for at least 7 days prior to transport to West Jefferson for study initiation.

(4) Acclimation -- All animals are held at the Medical Research and Evaluation Facility for at least 24 hours prior to study initiation.

(5) Selection -- Animals selected after the minimum 7-day quarantine period are in good physical condition based on appearance. Rabbits are weighed and assigned to groups based on body weight.

(6) Animal Identification -- All animals are ear tagged to retain positive identification during animal handling and observations. Cage cards are color-coded by group.

(7) Housing -- Animals are housed individually in stainless steel, slotted cages equipped with automatic watering systems.

(8) Lighting -- Fluorescent lighting, light/dark cycle is 12 hours each per day.

(9) Temperature -- Maintained at 70F (±5F).

(10) Humidity -- Maintained at 50% (±10%).

(11) Diet -- Purina Certified Rabbit Chow pellets are available at all times. No contaminants are known to be present in the feed which would interfere or affect the results of the study.

(12) Water Supply -- Water is supplied from the public water system and given ad libitum. No contaminants are known to be present in the water which would affect the results of the study.

B. Test Material

British Anti-Lewisite (2,3-dimercapto-1-propanol) will be purchased from a commercial supplier. Dimercaprol Injection, USP is available from Hynson, Westcott & Dunning, Baltimore, MD in ampules containing 100 mg BAL with 200 mg benzyl benzoate in 700 mg peanut oil per ml formulation. Since this article is a commercially prepared product, test article characterization, such as identity, strength, quality, stability and purity, will not be performed by Battelle. Requirements for test article characterization will be met by retaining all pertinent information provided by the supplier/manufacturer.

C. Test Groups

The determination of the lethality of BAL in rabbits following intramuscular injection is divided into three distinct phases. Phase 1 is a range-finding effort to determine the doses for the Phase 2 study to determine the LD_{50} of BAL. Phase 3 is a replication of the LD_{50}, adjusting doses as necessary.

(1) Range-Finding -- The acute 14-day LD_{50} range-finding study of intramuscularly administered BAL is performed in 6 groups of rabbits (2 males/group) at doses bracketting the estimated LD_{50}

(24.8 mg/kg per injection) at 0.15 log increments. The test article is administered by multiple intramuscular injection (4 equal amounts) at 4-hour intervals using a constant formulation concentration of 100 mg BAL/ml. Injections are made to the gluteal region.. An additional group of 2 male rabbits is similarly administered only the vehicle.

Group	Number of Male Rabbits	Dosage (mg/kg) per Injection Period
1	2	0 (vehicle only)
2	2	12.4
3	2	17.5
4	2	24.8 (LD_{50})
5	2	35.0
6	2	49.4
7	2	69.8

(2) Lethality Study -- The definitive 14-day LD_{50} study is performed in at least 5 groups (but not more than 8 groups) of rabbits (8 males/group) at doses bracketting the estimated LD_{50} determined in the preliminary range-finding study. The test article is administered by multiple intramuscular injections (4 equal amounts) at 4-hour intervals using a constant formulation concentration (100 mg BAL/ml). An additional group of 8 male rabbits is similarly administered the vehicle, 20 percent benzyl benzoate and 80 percent peanut oil (w/w). The largest dosage volume used for test animals will be used for the controls.

Group	Number of Male Rabbits	Dosage (mg/kg)
1	8	0 (vehicle only)
2	8	*
3	8	*
4	8	*
5	8	*
6	8	*
7 (if needed)	8	*
8 (if needed)	8	*
9 (if needed)	8	*

(*) Exact dosage levels are based on results of the previous range-finding study. A sufficient number of groups are used

to determine an appropriate LD_{50} with confidence limits. All groups are treated during the same day to minimize daily experimental variation.

(3) Replication of Lethality Study -- The lethality study is repeated, adjusting doses as necessary to produce a valid LD_{50} with acceptable confidence intervals.

D. Study Preparation

(1) Animals -- One day prior to the start of the study, the hind quarters of each animal is clipped free of hair using a small animal clipper. This is done to visually assure appropriate dosage administration.

(2) Marking Test Sites -- Four areas for injection, each about one square centimeter, are marked on the gluteal region of each animal with a water-based ink.

E. Application of BAL

(1) BAL is injected using a disposable 1-ml tuberculin syringe.

(2) The intramuscular injections are spaced over the injection area so that a new site is picked each time.

(3) Each animal receives four equal injections of BAL or vehicle at 4-hour intervals. The time of administration is recorded for each animal.

(4) The injection sites of all animals are inspected after the last rabbit has been dosed at each dosing interval. The animals are housed individually for the remainder of the study. In the event ulceration of the injection site occurs, animal collars will be used to prevent rabbits from disturbing the region of inflammation. Supportive treatment will be administered if it does not interfere with experimental results. Severely ulcerated animals will be terminated as moribund.

F. Specific Procedures

(1) Exposure timing is controlled by one investigator who also maintains the laboratory notebook. A second investigator

administers injections and a third investigator maintains a supply of rabbits from the preparation area.

(2) All animals are inspected after test article administration.

(3) Observations are made for signs of toxicity at least once every hour after the start of dosing and for the remainder of the work day. Mortality is recorded on the morning of the day following exposure. The condition of survivors is also recorded. Daily individual observations, with morning and afternoon checks for physical signs of toxicity, are recorded for the remainder of the study. When possible, the onset and duration of signs are ascertained and described.

(4) All surviving animals are killed 14 days after dosage administration by an intravenous overdose injection of T-61.

7. Necropsy and Histopathology:

Gross post-mortem examinations will not be performed for any animals during the study. No tissues will be saved and all carcasses will be discarded.

8. Statistical Methods:

An LD_{50} calculation, slope, and 95% confidence interval are made based on the results of the 24-hour and 14-day survival data. The calculation is performed according to the procedure of Finney, Probit Analyses, 3rd Ed. (1971), or by other suitable techniques.

9. Records to be Maintained:

A. Compound inventory, specifications, and usage

B. Dosage preparation and administration

C. Animal data

D. Clinical observations

E. Mortality

F. Disposal records

Revised October 10, 1984

10. Reports:

A final report will be prepared and submitted within 30 days after completion of the task. It includes the following:

1. Signature page for key study individuals and their responsibilities
2. Experimental design
3. Animal supplier
4. Test animal selection criteria
5. Test material description and preparation
6. Treatment procedures
7. Description of clinical observations
8. Tabulation of response data by dose, including doses used to calculate approximate LD_{50}
9. Statistical analyses used
10. Discussion.

12. Study Approval:

A. For Battelle:

Ronald L. Joiner, Ph.D.
Study Director

October 16, 1984
Date

H. Hugh Harroff, D.V.M.
Chief Veterinarian

October 16, 1984
Date

B. For USAMRDC:

LTC Howard Johnson, D.V.M.
Sponsor Monitor

17 Oct 84
Date

Revised October 10, 1984

Tissue Distribution of Arsenic in the Rabbit Following Administration of Lewisite With and Without BAL Therapy

Study performed by Battelle Columbus Laboratories
505 King Avenue, Columbus, Ohio 43201

1. Study Director: Ronald L. Joiner, Ph.D.

2. Veterinarian: H. Hugh Harroff, Jr., D.V.M.

3. Sponsor: U.S. Army Medical Research and Development Command

4. Sponsor Monitor: LTC(P) Howard C. Johnson, USAMRICD

5. Objective:

To determine the tissue distribution of arsenic in rabbits after administration of Lewisite (L) with and without 2,3-dimercapto-1-propanol (BAL) therapy. The dose levels of Lewisite and BAL are selected from the results of preliminary LD50 studies of each substance in this species. Brain, spinal cord, liver, kidney, fat, blood, testis, injection site skin tissue and a normal skin sample adjacent to the injection site, and lung tissue arsenic levels are determined at 0 hours and at 4, 12, 24, 48, and 96 hours after Lewisite administration. BAL is administered in 4 equal dosages at 4-hour intervals, beginning 1 hour after administration of Lewisite.

6. Experimental Design:

A. Test System

Albino rabbits were chosen for this study on the basis on the extensive data base available for this species.

(1) Animals -- New Zealand white (albino) male rabbits, supplied by Kings Wheel Rabbitry, Mt. Vernon, Ohio.

(2) Initial Weight -- 2.0 to 4.0 kilograms.

(3) Quarantine -- Rabbits are held in isolation and observed for clinical illness for at least 7 days prior to transport to West Jefferson for study initiation.

(4) Acclimation -- All animals are held at the Medical Research and Evaluation Facility (MREF) for at least 24 hours prior to study initiation.

(5) Selection -- Animals selected after the minimum 7-day quarantine period are in good physical condition based on appearance. Rabbits are weighed and randomly assigned to groups based on body weight.

(6) Animal Identification -- All animals are ear tattooed to retain positive identification during animal handling and observations. Cage cards are color-coded by group.

(7) Housing -- Animals are housed individually in stainless steel, slotted metabolic cages equipped with automatic watering systems.

(8) Lighting -- Fluorescent lighting is used in a light/dark cycle of 12 hours each per day.

(9) Temperature -- Maintained at 70 F (±5 F).

(10) Humidity -- Maintained at 50% (±10%).

(11) Diet -- Purina Certified Rabbit Chow pellets are available at all times. No contaminants are known to be present in the feed that would interfere with the results of the study.

(12) Water Supply -- Water is supplied from the public water system and given ad libitum. No contaminants are known to be present in the water that would interfere with the results of the study.

(13) Laboratory Animal Welfare Practices -- Battelle's Animal Resources Facilities have been registered with the U.S. Department of Agriculture as a Research Facility (Number 31-21) since August 14, 1967, and are periodically inspected in accordance with the provisions of the Federal Animal Welfare Act. In addition, animals for use in research are obtained only from laboratory animal suppliers duly licensed by the USDA. Battelle's statement of assurance regarding the Department of Health and Human Services policy on humane care of laboratory animals was accepted by the Office of Protection from Research Risks, National Institutes of Health on August 27, 1973. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the

Care and Use of Laboratory Animals" (DHEW Publication No. (NIH) 78-23), and/or in the regulations and standards as promulgated by the Agricultural Research Service, USDA, Pursuant to the Laboratory Animal Welfare Act of August 24, 1966 as amended (P.L. 89-544 and P.L. 91-579).

(14) Accreditation -- On January 31, 1978, Battelle's Columbus Division received FULL ACCREDITATION of its animal-care program and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. The MREF is a part of the facilities granted full accreditation.

B. Test Materials

(1) Lewisite (dichloro-2-chlorovinylarsine) is supplied by the USAMRDC/ICD. Purity, appropriate identification (batch number, lot number, state), and stability data are supplied by the USAMRDC/ICD. Purity and stability are confirmed periodically for materiel stored at Battelle.

(2) British Anti-Lewisite (2,3-dimercapto-1-propanol, BAL) will be purchased from a commercial supplier. BAL is available from Hynson, Westcott & Dunning, Baltimore, MD in a research grade that is listed as greater than 98% pure. Since this article is a commercially prepared product, test article characterization, such as identity, strength, quality, stability and purity, will not be performed by Battelle. Requirements for test article characterization will be met by retaining all pertinent information provided by the supplier/manufacturer.

(3) Samples of feed, drinking water, euthanasia agent, anesthetic agents, and other materials either fed or injected into test animals are assayed for arsenic content by atomic absorption spectrophotometry.

(4) Surety, security, and safety procedures for the use of CSM are thoroughly outlined in facility plans, in personnel requirements for qualifications to work with CSM, and in CSM storage and use standard operating procedures.

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Revised March 1, 1985

C. Test Groups

(1) For this study, 2 series of 100 rabbits each are administered Lewisite by subcutaneous injection - Series 1 at 3.5 mg/kg (approximately the LD40 dosage) and Series 2 at 2.4 mg/kg (approximately the LD10 dosage). These dosages are determined from preliminary range-finding and definitive LD50 studies. One hour following Lewisite treatment, one-half of the animals in each series will receive BAL therapy. This therapy consists of the administration of 140 mg/kg of BAL in 4 equal intramuscular injections of 35 mg/kg of BAL at 4-hour intervals. The 35 mg/kg dosage of BAL (approximately the LD01 dosage) was determined from preliminary range-finding and definitive LD50 studies in rabbits.

Five surviving rabbits in each Lewisite series (with and without BAL therapy) are sacrificed at 4, 12, 24, 48, and 96 hours after administration of Lewisite. At each sacrifice period, selected tissues (brain, spinal cord, liver, kidney, body fat, blood, testis, and lung) are removed for determination of tissue arsenic concentration. In addition, baseline tissue arsenic levels are determined in 5 rabbits given the ethanol vehicle only at the 0- and 96-hour sacrifice periods. Additional rabbits surviving to 96 hours are sacrificed without tissue retention.

(2) To facilitate animal handling, treatment, and tissue collection, the study is conducted in two parts:

(a) Part 1 consists of administering the LD10 dose of Lewisite to 50 rabbits to be sacrificed as described in the table below and to an additional 50 rabbits that receive BAL therapy and are then sacrificed as given below. Vehicle controls are also included.

(b) Part 2 repeats the study in Part 1 at the LD40 dose of Lewisite.

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PART I

Dose	Total Rabbits Dosed	Rabbits Sacrificed at Each Interval: 0 Hr.	4 Hr.	12 Hr.	24 Hr.	48 Hr.	96 Hr.	Sacrifice Total
2.4 mg/kg L only	50	--	5	5	5	5	5	25
2.4 mg/kg L plus 35 mg/kg BAL	50	--	5	5	5	5	5	25
Vehicle Control	10	5	--	--	--	--	5	10

PART II

3.5 mg/kg L only	50	--	5	5	5	5	5	25
3.5 mg/kg L plus 35 mg/kg BAL	50	--	5	5	5	5	5	25
Vehicle Control	10	5	--	--	--	--	5	10
Total	220	10	20	20	20	20	30	120

(3) All groups in each part of the study are treated during the same day to minimize daily experimental variation. Lewisite administration is by subcutaneous injection to the dorsal surface (back) in a region mid-way between the shoulders and the rump. This test article is suspended in ethanol and administered at a volume of 0.033 ml/kg body weight. Animals in the Lewisite/BAL therapy groups are administered BAL in ethanol (volume of 0.067 ml/kg body weight per dose) by intramuscular injection to the hind quarters. Four equal doses of BAL are administered at 4-hour intervals, beginning one hour after Lewisite treatment. Control animals receive a volume of ethanol equivalent to the vehicle

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volume for their weight (0.033 ml/kg). At the indicated time points, 5 surviving rabbits in the treated groups are randomly selected by animal identification number from the pool of surviving animals for sacrifice to obtain tissues for determination of arsenic concentration.

D. Study Preparation

(1) Animals -- One day prior to the start of the study, the back of each animal is clipped free of hair from the shoulders to and including the hind quarters with a small animal clipper. This is done to visually ensure appropriate dosage administration and to facilitate decontamination of the Lewisite injection site.

(2) Anesthesia -- Rabbits are given anesthetic doses (usually 17.5 mg/kg and 10 mg/kg, respectively) of a Rompun/Ketamine mixture (3.5 to 1, v/v) by intramuscular injection.

(3) Marking Test Sites -- Rabbits are placed in a metal restraining box to restrict movement. Four areas for BAL injection, each about one square centimeter, are marked on the quadriceps region of each animal to receive BAL therapy.

E. Application of Lewisite

(1) The subcutaneous Lewisite injections are administered by first lifting the skin from the musculature and then piercing the skin with the syringe needle.

(2) Each animal receives a single bolus injection of Lewisite.

(3) The time of administration is recorded for each animal.

(4) All dosages are administered while the animals are in an approved chemical fume hood.

F. Decontamination Procedures

(1) Following dose administration, the area of Lewisite injection is decontaminated with a 5% sodium hypochlorite solution on a gauze pad. The injection site is then blotted dry with plastic-backed absorbent toweling.

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(2) The Lewisite injection site of all animals is inspected after the last rabbit has been dosed. Animals are kept in the restrainers in the fume hood for at least 10 minutes after Lewisite injection to observe for seepage from the injection site. After that time, they are again decontaminated with 5% sodium hypochlorite followed by three distilled water rinses. Decontaminated animals can be removed from the hood and returned to stainless steel metabolic holding cages where they are housed for the remainder of the study.

(3) In the event ulceration of the injection site occurs, animal collars will be used to prevent rabbits from disturbing the region of inflammation. Supportive treatment will be administered if it does not interfere with experimental results. Severely ulcerated animals will be terminated as moribund.

G. BAL Administration

(1) BAL in ethanol is administered by intramuscular injection to the quadriceps region. Therapy consists of 4 equal doses administered to new injection sites at 4-hour intervals.

(2) The injection sites are marked with a water-based ink prior to dosage administration.

(3) Dosing begins one hour after Lewisite administration. The time of each dosage administration is recorded for each animal.

H. Observations

(1) Observations are made for mortality and signs of toxicity at least twice during the first day of exposure.

(2) Mortality is recorded on the morning of the day following exposure and daily thereafter. The condition of survivors is also recorded.

(3) Daily individual observations, with morning and afternoon checks for physical signs of toxicity, are recorded for the remainder of the study.

(4) Clinical observations are also recorded at the time of sacrifice of each animal.

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(5) All surviving animals are euthanized 4 days after dosage administration by an intravenous overdose injection of T-61.

7. Necropsy and Tissue Collection:

Gross post-mortem examinations are performed and the results recorded for any animals that spontaneously die (i.e., are not sacrificed) during the study; their tissues are not saved and their carcasses are discarded.

All animals designated for tissue distribution studies of arsenic (120 males) are euthanized with T-61 at appropriate time intervals. Samples of brain, spinal cord, liver, kidney, body fat, blood (5 ml), testes, and lung are begun being harvested within 5 minutes after sacrifice. In addition, tissue samples are taken from the injection site and from an area adjacent to the injection site but otherwise considered normal skin tissue. Portions of all harvested tissues (except blood, fat, and spinal cord) are trimmed, weighed, and preserved in 10 percent neutral buffered formalin for possible histopathologic evaluation. The remaining portions of the collected tissues are stored frozen at approximately -20 C for tissue arsenic concentration determinations. The remaining tissues and the carcasses are discarded.

8. Tissue Arsenic Determinations:

All tissue samples collected from designated treated and control animals are individually assayed for arsenic content, using flameless atomic absorption spectrographic techniques.

A. Tissue Storage

(1) All glassware and equipment used in collecting samples for arsenic analysis are first washed with dilute nitric acid and distilled water (DH_2O).

(2) Tissue samples are prepared for storage within 3 hours of sacrifice.

(3) Tissues are homogenized in a Waring blender, replaced in the same trace-element free container, and stored frozen at -20 C until analysis.

(4) The blender is cleaned between samples with a dilute nitric acid rinse, followed by three DH_2O rinses.

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(5) Whole blood is collected in vacutainer tubes containing sodium citrate buffer and stored frozen at -20 C in the same container until analysis.

B. Tissue Preparation

(1) After thawing, homogenized tissue is divided into 1-gm aliquots.

(2) Samples are digested with 2 ml of concentrated nitric acid, 1 ml of sulfuric acid, and 0.2 ml of magnesium nitrate solution (50 gm/100 ml).

(3) Samples are slowly heated until fuming begins, at which point 1 ml of 30% hydrogen peroxide is added.

(4) This procedure is repeated until sample solutions are clear, at which time the sample solutions are heated to dryness on a hot plate.

C. Tissue Analysis

(1) The reaction residue is dissolved in 20 ml of an acidic mixture containing potassium iodide (11.6 g/l), sodium ascorbate (1.4 g/l), and hydrochloric acid (250 ml/l).

(2) A 15-ml aliquot of the dissolved residue is placed into the reaction vessel of a mercury hydride generation system (Perkin-Elmer 603, MS-10).

(3) Arsine gas (AsH_3) is formed by sodium borohydride reduction in the hydride generation vessel by adding approximately 2 ml of a 2.5% sodium hydroxide and 5% sodium borohydride solution.

(4) The reaction vessel is purged with nitrogen and the arsine gas is transported to a Perkin-Elmer atomic absorption spectrophotometer equipped with an arsenic electrodeless discharge lamp operated at 193.7 nm.

(5) Peak heights are used for the calculation of the arsenic concentrations in the specimens.

(6) Blanks and standards are treated identically to the tissue samples.

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9. Statistical Methods:

The results from the arsenic analysis for each tissue are compared statistically in the following manner. Average values are determined for each series of animals sacrificed at each time period in each of the two regimens (Lewisite alone and Lewisite with BAL treatment). These average concentrations of arsenic (weight per gram of wet tissue) are compared with other average values at all other time periods in the same regimen (i.e., at 4, 12, 24, 48, and 96 hours) and with the average values of the two regimens at the same time period (i.e., Lewisite alone at 24 hours versus Lewisite plus BAL at 24 hours). In addition, average values from all Lewisite-injected animals (with and without BAL treatment) are compared to the average values of the vehicle controls collected at 0 and 96 hours.

Differences between and among these comparison groups are tested by one-way analysis of variance (ANOVA). Specific treatment versus control differences are determined by the least significant difference test.

If significant heterogeneity of variance is shown across the sacrifice groups of either regimen by the Bonferoni test, overall regimen comparisons may be made using the Kruskal-Wallis test, a non-parametric equivalent to the ANOVA. In this case, treatment versus control comparisons equivalent to the least significant different test may be made with a t-test using separate variance estimates for each comparison to be made.

10. Records to be Maintained:

A. Compound inventory, specifications, and usage

B. Dosage preparation and administration

C. Animal data (body weights, organ weights)

D. Arsenic analysis data (including diet, drinking water, etc.)

E. Clinical observations

F. Mortality

G. Proof of decontamination results and disposal records.

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11. Reports:

A draft final report will be prepared and submitted to the USAMRDC COTR within 30 working days after completion of the task. It includes at least the following:

1. Signature page for key study individuals and their responsibilities
2. Experimental Design
3. Animal supplier
4. Test animal selection criteria
5. Test material description and preparation
6. Treatment procedures
7. Description of clinical observations
8. Tabulation of tissue arsenic data by dose and sacrifice interval
9. Statistical analyses used
10. Discussion.

A final report that considers the review comments of the USAMRDC is prepared and submitted within 30 days of receipt of comments.

12. Study Approval:

Ronald L. Joiner, Ph.D.
Study Director — 1 April 1985 Date

H. Hugh Harroff, Jr., D.V.M.
Chief Veterinarian — " Date

LTC(P) Howard C. Johnson, D.V.M.
Sponsor Monitor — " Date

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13. Amendment A - May 22, 1985

This is to document several minor details for Protocol 12 (Tissue Distribution of Arsenic in the Rabbit Following Administration of Lewisite With and Without BAL Therapy).

1. Page 8, Section 8.A.(3)

Tissue samples are thawed and homogenized after storage at 20 C. Soft tissue samples weighing more than 1 gram are homogenized in a Waring commercial blender. A Polytron homogenizer is used to homogenize skin samples with distilled water that is analytically determined to be arsenic-free. Ten milliliters of distilled water is added to produce a liquid consistency that facilitates homogenization of the skin. Tissue samples weighing 1 gram or less (spinal cord section, testis) are not homogenized but are chemically digested *in toto* as detailed in Section 8.B.

14. Approval Signatures:

Ronald L. Joiner — 24 MAY 1985
Ronald L. Joiner, Ph.D. — Date
Study Director

H. Hugh Harroff, Jr. D.V.M. — 28 May 1985
H. Hugh Harroff, Jr., D.V.M. — Date
Chief Veterinarian

Howard C. Johnson — 24 MAY 85
LTC(P) Howard C. Johnson, D.V.M. — Date
Sponsor Monitor

Revised October 10, 1984
Revised March 1, 1985

APPENDIX B

METHOD DEVELOPMENT FOR DETECTION OF ARSENIC IN THE RABBITS BY ATOMIC ABSORPTION

METHOD DEVELOPMENT FOR DETECTION OF ARSENIC IN THE RABBIT BY ATOMIC ABSORPTION

(G8180-1400)

by

K. McNeill, A. Wensky, D. Sgontz, and G. Fisher

METHOD DEVELOPMENT FOR DETECTION OF ARSENIC IN THE RABBIT BY ATOMIC ABSORPTION

A sensitive method of analysis to determine the tissue distribution of arsenic in rabbits after administration of Lewisite (L) with and without BAL therapy was needed for evaluation of the efficacy of antidotal compounds. To that end, a pilot study was used to evaluate current techniques for arsenic detection. Two earlier studies (1,2), which analyzed arsenic in rat and hamster tissues using a hydride generation system with atomic absorption, described the basic methodology used in the study. The use of a hydride generator in these earlier publications increased the sensitivity of arsenic detection. Further refinements detailed in the appended protocol were necessary to quantitatively analyze the low levels of arsenic in rabbit tissues. Sample preparation was modified to detect the arsenic from samples without significant loss.

The method of arsenic analysis developed for this study was evaluated for sensitivity and reproducibility by analysis of multiple samples of tissue derived from one rabbit. Tables 1-3 present the arsenic levels found in spiked and unspiked samples in brain, whole blood, and liver. Arsenic was not detected by atomic absorption (detection limit <5 ng/g) in the unspiked blood or brain samples. Liver arsenic concentrations were 6 ng/g, which is in agreement with work done using neutron activation analysis by Marafante et al. (3). Analysis of blood and brain tissue from the same study (3) was 3 and 1 ng/g, respectively.

The spiked samples displayed good recovery of inorganic and organic arsenic and were quantitative within a range of 20-40 ng/g wet tissue. Spike recovery was calculated after subtracting the background level of arsenic detected for that tissue from the amount of arsenic spiked. The inorganic spike recovery was somewhat greater than the organic and this discrepancy was unexplained. In general, sample reproducibility was good with the exception of two unspiked liver samples (Table 3). These two higher values indicated a possible arsenic contamination after the homogenized tissue had been aliquoted into individualized samples, because all other liver values were in agreement.

Tissue distribution of arsenic was determined from rabbits treated with L to further evaluate the methodology developed for arsenic analysis. Rabbits received L or vehicle only and were sacrificed as they became moribund. A control rabbit was sacrificed 72 hours after exposure to match a 4.2 mg/kg dosed animal terminated at that time; a second control rabbit was sacrificed with two rabbits which received 4.2 or 2.9 mg/kg of L 96 hours earlier. Whole blood, brain, and kidneys from each rabbit were prepared for analysis using the appended procedures.

Table 4 presents the arsenic levels detected in brain, whole blood, and kidney from control and dosed rabbits. Arsenic was not detected in the brain or blood from control rabbits and was found in very low levels (12-15 ng/g) in the kidneys of both controls. Marafante et al. (3) found 6.5 ng/g of arsenic in the kidneys from untreated rabbits by neutron activation analysis. A third sample from one control animal was spiked with inorganic arsenic and after subtracting the background arsenic level, displayed good recovery of 110, 112, and 105 percent of the spike for brain, blood, and kidney, respectively. Duplicate samples were run on one control and one dosed animal. The analysis of duplicate samples from the dosed animal (2.9 mg/kg of L) demonstrated good sample agreement.

There was little inter-animal variation seen in the tissue arsenic concentrations from the two rabbits administered 4.2 mg/kg of L (Table 4). Arsenic concentrations in the brain of each animal were 710 and 630 ng/g, blood values were 340 and 320 ng/ml, and kidney concentrations were 2600 and 2400 ng/g, respectively.

Table 5 presents the percent of the total arsenic dose found in the tissues analyzed. The two rabbits administered L at 4.2 mg/kg (Nos. 291 and 338) had similar patterns of arsenic distribution even though there was a 24-hour interval between the sacrifice of the first and second animal. It was encouraging to detect a readily quantifiable amount of arsenic in tissues from rabbits 96 hours after an acute dose of L. The sensitivity in the detection limit coupled with good spike recoveries confirmed that the current methodology was adequate for detection of low levels of arsenic in the tissues from rabbits.

Protocol for Arsenic Analysis

Tissue Preparation

Tissue samples were received within 3 hours of sacrifice in trace element-cleaned glass bottles. Tissues (brain, liver, or kidney) were homogenized in a Waring blender, replaced in the same container and stored frozen (-20 C) until use. The blender was cleaned between samples with a dilute nitric rinse followed by three DH_2O rinses. Whole blood was collected in vacutainer tubes containing sodium citrate buffer and stored frozen in the same container until analysis.

Tissue Analysis

After thawing, homogenized tissue was divided into 1-g aliquots and the weights recorded. Samples were digested with 2 ml of concentrated HNO_3, 1 ml of H_2SO_4, and 0.2 ml of $Mg(NO_3)_2$ solution (50 g/100 ml). Samples were slowly heated until fuming began, at which point 1 ml of 30 percent H_2O_2 was added. This procedure was repeated until sample solutions were clear. The sample solutions were then brought to dryness on a hot plate.

The reaction residue was dissolved in 20 ml of an acid mixture (11.6 g/L KI, 1.4 g/L Na Ascorbate, 250 ml/L HCl). A 15-ml aliquot of the dissolved residue was put into the reaction vessel of a Hg hydride system (Perkin-Elmer 603, MS-10). AsH_3 was formed by sodium borohydride reduction in the hydride generation vessel by adding approximately 2 ml of a 2.5 percent NaOH and 5 percent sodium borohydride solution. The reaction vessel was purged with nitrogen and the AsH_3 gas was transported to a Perkin-Elmer atomic absorption spectrophotometer equipped with an arsenic electrodeless discharge lamp operated at 193.7 nm. Peak heights were used for the calculation of the As concentrations in the specimens. The blanks and standards were treated identically to the tissue samples.

References

1. G. Pershagen, B. Lind, and N. Bjorklund. Lung Retention and Toxicity of Some Inorganic Arsenic Compounds. Environ. Res. 29:425-434, 1982.

2. S. Valkoven, H. Savolainen, and J. Jarvisalo. Arsenic Distribution and Neurochemical Effects in Peroral Sodium Arsenite Exposure of Rats. Bull. Environ. Contam. Toxicol. 30:303-308, 1983.

3. E. Marafante, F. Bertolero, J. Edel, R. Pietra, and E. Sabbioni. Intracellular Interaction and Biotransformation of Arsenite in Rats and Rabbits. Sci. Total. Environ. 24:27-39, 1982.

TABLE 1. ARSENIC IN RABBIT BRAIN

Sample No.	Weight (g)	Amount Found (PPB)	Amount Spiked (PPB)	Spike Recovery (%)	As Type Spiked
1	0.978	<5	--	--	--
2	1.065	<5	--	--	--
3	1.068	21	23	91	Organic
4	0.949	21	26	81	Organic
5	1.054	30	24	125	Inorganic
6	1.037	29	24	121	Inorganic

--Sample not spiked.

TABLE 2. ARSENIC IN RABBIT BLOOD

Sample No.	Weight (g)	Amount Found (PPB)	Amount Spiked (PPB)	Spike Recovery (%)	As Type Spiked
1	1.076	<5	--	--	--
2	1.060	<5	--	--	--
3	1.059	<5	--	--	--
4	1.040	19	24	79	Organic
5	1.023	20	24	83	Organic
6	1.034	42	48	88	Organic
7	1.032	52	48	108	Organic
8	1.034	28	24	117	Inorganic
9	1.028	26	24	108	Inorganic
10	1.019	59	49	120	Inorganic
11	1.030	54	49	110	Inorganic

--Sample not spiked.

TABLE 3. ARSENIC IN RABBIT LIVER

Sample No.	Weight (g)	Amount Found (PPB)	Amount Spiked (PPB)	Spike Recovery (%)	As Type Spiked
1	1.022	6	--	--	--
2	1.012	6	--	--	--
3	1.055	46	--	--	--
4	1.093	41	--	--	--
5	1.009	6	--	--	--
6	1.108	6	--	--	--
7	1.000	26	25	80	Organic
8	1.680	26	23	87	Organic
9	1.028	51	49	92	Organic
10	1.112	51	45	100	Organic
11	1.016	38	25	128	Inorganic
12	1.021	30	24	100	Inorganic
13	1.089	58	46	113	Inorganic
14	1.020	70	49	131	Inorganic

*Background As subtracted before calculating spike recovery.
--Sample not spiked.

TABLE 4. ARSENIC DISTRIBUTION IN TISSUES FROM RABBITS DOSED WITH LEWISITE

Tissue	I.D.	Dose (mg/kg)	Weight (g)	As content (ng/g) As detected	As content (ng/g) Spike (inorg. As)	% Spike Recovery **
Brain	388	0*	1.085	<5	0	
	388	0*	1.000	<5	0	
	388	0	1.024	54	49	110
	390	0	1.065	<5	0	
	325	2.9*	0.972	370	0	
	325	2.9*	1.101	360	0	
	291	4.2	1.095	710	0	
	338	4.2	1.097	630	0	
Whole	388	0*	1.048	<5	0	
Blood	388	0*	1.025	<5	0	
	388	0	1.019	55	49	112
	390	0	1.020	<5	0	
	325	2.9*	1.044	120	0	
	325	2.9*	1.028	130	0	
	291	4.2	1.062	340	0	
	338	4.2	1.029	320	0	
Kidney	388	0*	1.145	14	0	
	388	0*	1.000	15	0	
	388	0	1.043	65	48	105
	390	0	1.091	12	0	
	325	2.9*	1.069	1200	0	
	325	2.9*	1.079	1100	0	
	291	4.2	1.036	2600	0	
	338	4.2	1.108	2400	0	

*Duplicate samples.
**Background As subtracted before calculating spike recovery.

TABLE 5. ARSENIC DISTRIBUTION IN SELECTED TISSUES FROM RABBITS DOSED WITH LEWISITE

I.D.	Total Lewisite Dose (mg)	Total As Dose (mg)	Time After Dose (hr)	% of Total As Dose		
				Whole Blood	Brain	Kidney
388	0	0	96	0	0	0
390	0	0	72	0	0	0
325	6.6	2.4	96	0.8	0.13	1.0
291	8.5	3.1	96	1.6	0.18	1.8
338	9.6	3.5	72	1.5	0.16	1.4

APPENDIX C

Tables

TABLE 3.1.1. MORTALITY PROFILE OF RABBITS GIVEN SUBCUTANEOUS DOSES OF L IN A RANGE-FINDING STUDY

Dose (mg/kg)	Number Dosed	Number of Deaths Day 1	2	3	4	5	6	7	8	9	Total Deaths
(December 13, 1984)											
0.8	4	0	0	0	0	0	0	0	0	0	0
1.3	4	0	0	0	0	0	2	0	0	0	2
2.0	4	0	0	0	0	0	1	0	0	0	1
3.2	4	0	3	0	0	0	0	0	0	0	3
5.0	4	0	4	0	0	0	0	0	0	0	4

TABLE 3.1.2. MORTALITY PROFILE OF RABBITS GIVEN SUBCUTANEOUS DOSES OF L

Dose (mg/kg)	Number Dosed	Number of Deaths by Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Deaths
Replicate 1 (January 23, 1985 and February 1, 1985)																
0.8	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.3	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.4	8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
2.9	8	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2
3.2	8	0	0	1	0	1	1	0	1	0	0	0	0	0	0	4
3.5	8	0	2	0	0	0	0	0	0	0	0	0	1	0	1	4
4.2	8	0	0	1	1	2	1	0	0	0	0	0	0	0	0	5
5.0	8	1	2	0	1	0	0	0	1	0	0	1	0	0	0	6
5.0	8	0	1	0	2	1	0	2	1	0	0	0	0	0	0	7
Replicate 2 (February 14, 1985)																
2.0	8	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
2.4	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.9	8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
3.5	8	0	2	0	0	1	0	0	0	1	0	0	0	0	0	4
4.2	8	0	2	0	0	0	0	1	0	0	0	0	0	0	0	3
5.0	8	0	3	0	0	1	0	2	0	0	0	0	0	0	0	6

TABLE 3.1.3. MORTALITY PROFILE OF RABBITS GIVEN FOUR INTRAMUSCULAR DOSES OF BAL IN TWO RANGE-FINDING STUDIES

Dose Per Injection	Total Dose (mg/kg)	Number Dosed	Number of Deaths Day 1	2	3	4	5	6	7	8	9	Total Deaths
(December 4, 1984)												
0.0	0.0	2	0	0	0	0	0	0	0	0	0	0
12.4	49.6	2	0	0	0	0	0	0	0	0	0	0
17.5	70.0	2	0	0	0	0	0	0	0	0	0	0
24.8	99.2	2	0	0	0	0	0	0	0	0	0	0
35.0	140.0	2	0	0	0	0	0	0	0	0	0	0
49.4	197.6	2	1	0	0	0	0	0	0	0	0	1
69.8	279.2	2	2	0	0	0	0	0	0	0	0	2
(January 3, 1985)												
17.5	70.0	2	0	0	0	0	0	0	0	0	0	0
22.1	88.4	2	0	0	0	0	0	0	0	0	0	0
27.8	111.2	2	0	1	0	0	0	0	0	0	0	1
35.0	140.0	2	0	0	0	0	0	0	0	0	0	0
44.1	176.4	2	0	0	1	1	0	0	0	0	0	2
55.5	222.0	2	1	0	0	0	1	0	0	0	0	2
69.8	279.2	2	1	1	0	0	0	0	0	0	0	2

TABLE 3.1.4. MORTALITY PROFILE OF RABBITS GIVEN FOUR INTRAMUSCULAR DOSES OF BAL

Dose Per Injection (mg/kg)	Total Dose (mg/kg)	Number Dosed	Number of Deaths Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Deaths
Replicate 1 (January 16 and 30, 1985)																	
12.4	49.6	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.5	70.0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.8	99.2	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35.0	140.0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.4	197.6	8	1	4	0	1	0	0	0	0	0	0	0	0	0	0	6
69.8	279.2	8	7	1	0	0	0	0	0	0	0	0	0	0	0	0	8
35.0	140.0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40.2	160.8	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46.1	184.4	8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
53.0	212.0	8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2
60.8	243.2	8	3	3	0	0	0	0	0	0	0	0	0	0	0	0	6
69.8	279.2	8	7	1	0	0	0	0	0	0	0	0	0	0	0	0	8
Replicate 2 (February 20, 1985)																	
47.6	190.4	8	0	1	0	0	0	0	1	0	0	0	0	0	0	0	2
50.6	202.4	8	0	2	0	0	0	1	0	0	0	0	0	0	0	0	3
53.9	215.6	8	1	5	0	0	0	0	0	0	0	0	0	0	0	0	6
57.3	229.2	8	4	1	1	0	0	0	0	0	0	0	0	0	0	0	6
61.0	244.0	8	3	4	0	0	0	0	0	0	0	0	0	0	0	0	7
65.0	260.0	8	6	1	0	0	0	0	0	0	0	0	0	0	0	0	7

TABLE 3.1.5. MEDIAN 14-DAY LETHALITY VALUES (mg/kg) IN RABBITS FOR SUBCUTANEOUS INJECTION OF L OR FOR INTRAMUSCULAR INJECTIONS OF BAL

Treatment	N	LD_{50}	LL	UL	Slope ± 2SE
		LEWISITE			
Replicate 1	88	3.61	3.21	4.13	7.05
Replicate 2	48	4.13	3.47	6.00	5.45
Composite	136	3.79	3.44	4.25	6.39 ± 2.17
		BAL			
Replicate 1	96	52.5*	49.2	56.3	16.0
Replicate 2	48	51.8*	45.7	55.1	14.9
Composite	144	52.2*	49.8	54.5	15.8 ± 5.4

N = Number of rabbits
LL = Lower 95 percent confidence limit
UL = Upper 95 percent confidence limit
SE = Standard error
* = Single injection dose in a regimen of four doses; i.e., the LD_{50} value for BAL is four times the value given here for the single injection dose.

TABLE 3.1.6. DOSE LEVELS (mg/kg) CALCULATED AND SELECTED FOR L AND BAL ADMINISTRATION IN RABBITS FOR THE TISSUE ARSENIC DISTRIBUTION STUDIES

Treatment	Calculated Levels			Rounded For Dosing
	Dose	LL	UL	
	LEWISITE			
LD10	2.39	1.92	2.71	2.4
LD40	3.46	3.12	3.82	3.5
	BAL			
LD01	37.2*	30.8	41.0	35.0

LL = Lower 95 percent confidence limit
UL = Upper 95 percent confidence limit
* = Single injection dose in a regimen of four doses; i.e., the LD_{01} value for BAL is four times the value given here for the single injection dose.

TABLE 3.2.1. RABBIT BRAIN WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Brain Weight (g)	Animal Number	Brain Weight (g)	Animal Number	Brain Weight (g)
0					B1231	8.59
0					B1315	9.51
0					B1412	8.76
0					B1423	8.73
0					B1441	7.59
4	B1319	9.38	B1367	7.91		
4	B1394	9.34	B1373	8.50		
4	B1430	8.62	B1375	9.21		
4	B1437	8.94	B1389	8.10		
4	B1450	9.23	B1416	8.15		
12	B1374	9.07	B1316	9.19		
12	B1404	9.51	B1363	8.66		
12	B1422	8.18	B1395	8.47		
12	B1442	8.71	B1400	8.86		
12	B1444	9.14	B1449	8.76		
24	B1352	8.20	B1318	8.92		
24	B1358	12.32*	B1332	8.86		
24	B1378	8.49	B1387	8.42		
24	B1420	8.54	B1421	8.58		
24	B1439	9.57	B1424	8.65		
48	B1312	9.18	B1205	7.83		
48	B1356	9.02	B1354	8.66		
48	B1379	9.17	B1362	8.50		
48	B1386	8.51	B1369	8.75		
48	B1440	8.58	B1397	8.37		
96	B1196	9.15	B1357	8.76	B1314	8.67
96	B1381	8.18	B1383	9.33	B1364	7.72
96	B1405	8.97	B1392	8.76	B1411	8.30
96	B1419	8.24	B1434	8.92	B1418	8.86
96	B1428	8.62	B1438	8.83	B1443	8.54

*Outlier as determined by two-sided outlier test at alpha = 0.0026 ($\pm$3 standard deviations).

TABLE 3.2.2. RABBIT LUNGS WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Lungs Weight (g)	Animal Number	Lungs Weight (g)	Animal Number	Lungs Weight (g)
0					B1231	26.53
0					B1315	22.26
0					B1412	10.30
0					B1423	26.32
0					B1441	17.17
4	B1319	9.03	B1367	9.25		
4	B1394	25.74	B1373	11.70		
4	B1430	10.07	B1375	11.96		
4	B1437	9.95	B1389	10.57		
4	B1450	21.36	B1416	13.66		
12	B1374	11.01	B1316	10.77		
12	B1404	12.28	B1363	13.56		
12	B1422	12.12	B1395	25.91		
12	B1442	9.20	B1400	11.18		
12	B1444	8.66	B1449	9.54		
24	B1352	9.30	B1318	30.71		
24	B1358	8.96	B1332	27.16		
24	B1378	8.98	B1387	9.15		
24	B1420	10.33	B1421	37.74		
24	B1439	10.16	B1424	26.46		
48	B1312	12.89	B1205	9.67		
48	B1356	16.21	B1354	11.42		
48	B1379	13.55	B1362	13.99		
48	B1386	19.50	B1369	26.94		
48	B1440	20.52	B1397	14.71		
96	B1196	27.58	B1357	19.92	B1314	9.75
96	B1381	9.28	B1383	27.01	B1364	23.55
96	B1405	22.54	B1392	13.31	B1411	35.28
96	B1419	11.35	B1434	10.12	B1418	29.98
96	B1428	13.22	B1438	23.26	B1443	15.49

TABLE 3.2.3. RABBIT LIVER WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Liver Weight (g)	Animal Number	Liver Weight (g)	Animal Number	Liver Weight (g)
0					B1231	98.22
0					B1315	93.50
0					B1412	89.93
0					B1423	135.69
0					B1441	98.47
4	B1319	143.66	B1367	102.08		
4	B1394	80.72	B1373	96.06		
4	B1430	61.03	B1375	101.36		
4	B1437	123.62	B1389	111.55		
4	B1450	107.08	B1416	125.95		
12	B1374	97.67	B1316	127.79		
12	B1404	129.68	B1363	121.57		
12	B1422	116.25	B1395	76.25		
12	B1442	69.86	B1400	118.87		
12	B1444	121.79	B1449	156.09		
24	B1352	122.15	B1318	128.93		
24	B1358	92.73	B1332	101.59		
24	B1378	86.33	B1387	114.64		
24	B1420	134.69	B1421	134.61		
24	B1439	93.93	B1424	105.49		
48	B1312	120.42	B1205	83.38		
48	B1356	125.43	B1354	102.12		
48	B1379	125.97	B1362	85.67		
48	B1386	94.12	B1369	82.79		
48	B1440	103.48	B1397	81.96		
96	B1196	125.95	B1357	86.76	B1314	156.20
96	B1381	107.79	B1383	82.31	B1364	124.85
96	B1405	162.79	B1392	-	B1411	118.87
96	B1419	144.20	B1434	82.81	B1418	122.80
96	B1428	98.48	B1438	91.85	B1443	124.51

-Weight not measured.

TABLE 3.2.4. RABBIT KIDNEYS WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Kidneys Weight (g)	Animal Number	Kidneys Weight (g)	Animal Number	Kidneys Weight (g)
0					B1231	16.76
0					B1315	17.21
0					B1412	15.59
0					B1423	15.98
0					B1441	16.86
4	B1319	17.90	B1367	12.44		
4	B1394	14.49	B1373	11.87		
4	B1430	12.68	B1375	15.24		
4	B1437	15.38	B1389	15.64		
4	B1450	16.15	B1416	14.14		
12	B1374	15.02	B1316	17.28		
12	B1404	24.14	B1363	16.39		
12	B1422	15.09	B1395	15.56		
12	B1442	13.94	B1400	16.90		
12	B1444	18.43	B1449	19.73		
24	B1352	17.67	B1318	26.46		
24	B1358	16.09	B1332	20.74		
24	B1378	13.51	B1387	19.64		
24	B1420	19.16	B1421	19.01		
24	B1439	16.35	B1424	17.45		
48	B1312	19.02	B1205	13.96		
48	B1356	16.95	B1354	18.48		
48	B1379	18.37	B1362	13.43		
48	B1386	17.33	B1369	15.26		
48	B1440	13.77	B1397	13.82		
96	B1196	21.72	B1357	20.36	B1314	20.99
96	B1381	12.54	B1383	14.03	B1364	15.80
96	B1405	16.53	B1392	13.11	B1411	14.37
96	B1419	16.36	B1434	14.19	B1418	19.03
96	B1428	16.39	B1438	14.67	B1443	14.90

TABLE 3.2.5. RABBIT TESTES WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Testes Weight (g)	Animal Number	Testes Weight (g)	Animal Number	Testes Weight (g)
0					B1231	1.94
0					B1315	3.44
0					B1412	1.51
0					B1423	1.47
0					B1441	1.20
4	B1319	3.22	B1367	0.93		
4	B1394	2.13	B1373	1.96		
4	B1430	1.82	B1375	1.64		
4	B1437	1.18	B1389	1.52		
4	B1450	2.12	B1416	1.19		
12	B1374	1.97	B1316	2.30		
12	B1404	1.58	B1363	0.70		
12	B1422	3.45	B1395	1.13		
12	B1442	0.69	B1400	2.21		
12	B1444	1.65	B1449	0.77		
24	B1352	1.73	B1318	1.78		
24	B1358	1.34	B1332	3.77		
24	B1378	1.06	B1387	1.52		
24	B1420	1.59	B1421	3.36		
24	B1439	1.71	B1424	0.90		
48	B1312	2.57	B1205	1.50		
48	B1356	1.17	B1354	2.95		
48	B1379	1.27	B1362	1.27		
48	B1386	1.27	B1369	2.49		
48	B1440	1.80	B1397	1.20		
96	B1196	2.38	B1357	0.63	B1314	1.76
96	B1381	0.70	B1383	1.34	B1364	1.90
96	B1405	1.89	B1392	0.80	B1411	2.18
96	B1419	1.83	B1434	1.25	B1418	1.91
96	B1428	0.85	B1438	1.65	B1443	1.96

TABLE 3.2.6. RABBIT DOSE-SITE SKIN WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	I L & BAL		II L Alone	
Nominal Sacrifice Time (Hrs) post-dosing)	Animal Number	Dose-Site Skin Wt (g)	Animal Number	Dose-Site Skin Wt (g)
4	B1319	14.24	B1367	23.13
4	B1394	14.51	B1373	16.03
4	B1430	10.81	B1375	17.54
4	B1437	14.73	B1389	17.60
4	B1450	9.36	B1416	18.02
12	B1374	15.93	B1316	21.35
12	B1404	15.89	B1363	19.89
12	B1422	11.02	B1395	16.61
12	B1442	8.30	B1400	34.69
12	B1444	17.14	B1449	15.76
24	B1352	18.75	B1318	18.76
24	B1358	25.37	B1332	35.76
24	B1378	7.34	B1387	26.13
24	B1420	13.92	B1421	26.57
24	B1439	8.38	B1424	38.16
48	B1312	21.24	B1205	20.50
48	B1356	8.85	B1354	14.13
48	B1379	18.60	B1362	24.23
48	B1386	20.55	B1369	21.84
48	B1440	16.92	B1397	19.64
96	B1196	15.55	B1357	10.23
96	B1381	8.65	B1383	17.43
96	B1405	11.95	B1392	18.13
96	B1419	9.56	B1434	28.06
96	B1428	13.90	B1438	27.89

Note: Dose-site skin weights for the vehicle control group are not presented, since lesions were not well defined at the dose site for these animals.

TABLE 3.2.7. GROUP MEAN (STANDARD DEVIATION) ORGAN WEIGHTS (g) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 2.4 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	8.4	(0.5)	8.8	(0.3)	8.7	(0.2)	8.4	(0.4)	8.9	(0.2)
	L & BAL	9.1	(0.3)	8.9	(0.5)	8.7	(0.6)	8.9	(0.3)	8.6	(0.4)
	Vehicle Only	8.6	(0.7)							8.4	(0.4)
Lungs*	L Alone	11.4	(1.6)	14.2	(6.7)	25.2	(10.6)	15.4	(6.8)	18.7	(7.0)
	L & BAL	15.2	(7.8)	10.7	(1.7)	9.6	(0.7)	16.5	(3.4)	16.8	(7.9)
	Vehicle Only	20.5	(6.9)							22.8	(10.4)
Liver	L Alone	107.4	(11.8)	120.1	(28.6)	117.1	(14.4)	87.2	(8.5)	85.9	(4.4)
	L & BAL	103.2	(33.0)	107.1	(23.9)	106.0	(21.2)	113.9	(14.3)	127.8	(26.3)
	Vehicle Only	103.2	(18.5)							129.5	(15.1)
Kidneys	L Alone	13.9	(1.7)	17.2	(1.6)	20.7	(3.5)	15.0	(2.1)	15.3	(2.9)
	L & BAL	15.3	(1.9)	17.3	(4.2)	16.6	(2.1)	17.1	(2.0)	16.7	(3.3)
	Vehicle Only	16.5	(0.7)							17.0	(2.9)
Testes	L Alone	1.5	(0.4)	1.4	(0.8)	2.3	(1.2)	1.9	(0.8)	1.1	(0.4)
	L & BAL	2.1	(0.7)	1.9	(1.0)	1.5	(0.3)	1.6	(0.6)	1.5	(0.7)
	Vehicle Only	1.9	(0.9)							2.1	(0.4)
Dose-Site Skin	L Alone	18.5	(2.7)	21.7	(7.6)	29.1	(7.9)	20.1	(3.7)	20.4	(7.6)
	L & BAL	12.7	(2.5)	13.7	(3.8)	14.8	(7.5)	17.2	(5.0)	11.9	(2.9)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other ($P<0.01$).
*See text for explanation.

TABLE 3.2.8. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT BLOOD FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Blood As (ng/g)	Animal Number	Blood As (ng/g)	Animal Number	Blood As (ng/g)
0					B1231	10
0					B1315	<10
0					B1412	32
0					B1423	29
0					B1441	19
4	B1319	826	B1367	566		
4	B1394	370	B1373	707		
4	B1430	543	B1375	537		
4	B1437	332	B1389	171		
4	B1450	312	B1416	374		
12	B1374	197	B1316	390		
12	B1404	159	B1363	225		
12	B1422	81	B1395	459		
12	B1442	111	B1400	433		
12	B1444	137	B1449	292		
24	B1352	74	B1318	169		
24	B1358	60	B1332	213		
24	B1378	79	B1387	175		
24	B1420	51	B1421	216		
24	B1439	80	B1424	191		
48	B1312	48	B1205	158		
48	B1356	40	B1354	114		
48	B1379	48	B1362	165		
48	B1386	44	B1369	206		
48	B1440	57	B1397	212		
96	B1196	33	B1357	91	B1314	21
96	B1381	56	B1383	96	B1364	26
96	B1405	36	B1392	63	B1411	20
96	B1419	43	B1434	114	B1418	18
96	B1428	36	B1438	85	B1443	35

TABLE 3.2.9. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT BRAIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Brain As (ng/g)	Animal Number	Brain As (ng/g)	Animal Number	Brain As (ng/g)
0					B1231	<6
0					B1315	10
0					B1412	<6
0					B1423	<5
0					B1441	<5
4	B1319	218	B1367	157		
4	B1394	171	B1373	231		
4	B1430	163	B1375	141		
4	B1437	133	B1389	29*		
4	B1450	25*	B1416	131		
12	B1374	100	B1316	206		
12	B1404	94	B1363	139		
12	B1422	55	B1395	155		
12	B1442	36	B1400	132		
12	B1444	62	B1449	150		
24	B1352	76	B1318	160		
24	B1358	44	B1332	174		
24	B1378	51	B1387	182		
24	B1420	103	B1421	153		
24	B1439	54	B1424	204		
48	B1312	29	B1205	160		
48	B1356	31	B1354	221		
48	B1379	21	B1362	189		
48	B1386	24	B1369	170		
48	B1440	60	B1397	232		
96	B1196	18	B1357	267	B1314	<7
96	B1381	24	B1383	216	B1364	<5
96	B1405	32	B1392	178	B1411	<7
96	B1419	24	B1434	205	B1418	<5
96	B1428	27	B1438	165	B1443	<6

*Outlier as determined by two-sided outlier test at alpha = 0.0026 ($\pm$3 standard deviations).

TABLE 3.2.10. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT SPINAL CORD FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Spinal Cord As (ng/g)	Animal Number	Spinal Cord As (ng/g)	Animal Number	Spinal Cord As (ng/g)
0					B1231	<12.0
0					B1315	18.0
0					B1412	-
0					B1423	<10.0
0					B1441	<30.0
4	B1319	287	B1367	108		
4	B1394	172	B1373	78		
4	B1430	224	B1375	85		
4	B1437	241	B1389	88		
4	B1450	178	B1416	65		
12	B1374	92	B1316	99		
12	B1404	100	B1363	151		
12	B1422	68	B1395	105		
12	B1442	-	B1400	82		
12	B1444	61	B1449	85		
24	B1352	48	B1318	101		
24	B1358	35	B1332	62		
24	B1378	40	B1387	97		
24	B1420	72	B1421	113		
24	B1439	50	B1424	253		
48	B1312	35	B1205	221		
48	B1356	38	B1354	120		
48	B1379	<25	B1362	64		
48	B1386	35	B1369	167		
48	B1440	61	B1397	149		
96	B1196	25	B1357	139	B1314	<15.0
96	B1381	29	B1383	106	B1364	<9.0
96	B1405	<17	B1392	104	B1411	<16.0
96	B1419	15	B1434	134	B1418	<6.5
96	B1428	18	B1438	105	B1443	<8.4

-Sample not analyzed.

TABLE 3.2.11. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT LUNG FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT B.L THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Lung As (ng/g)	Animal Number	Lung As (ng/g)	Animal Number	Lung As (ng/g)
0					B1231	12
0					B1315	24
0					B1412	17
0					B1423	61
0					B1441	28
4	B1319	524	B1367	4,827		
4	B1394	489	B1373	5,455		
4	B1430	2,192	B1375	402		
4	B1437	2,660	B1389	5,243		
4	B1450	957	B1416	3,104		
12	B1374	397	B1316	3,945		
12	B1404	331	B1363	1,593		
12	B1422	223	B1395	1,004		
12	B1442	399	B1400	2,152		
12	B1444	196	B1449	3,042		
24	B1352	662	B1318	513		
24	B1358	182	B1332	1,076		
24	B1378	346	B1387	2,041		
24	B1420	498	B1421	470		
24	B1439	383	B1424	501		
48	B1312	467	B1205	3,349		
48	B1356	134	B1354	966		
48	B1379	25	B1362	723		
48	B1386	179	B1369	639		
48	B1440	52	B1397	876		
96	B1196	53	B1357	697	B1314	9
96	B1381	170	B1383	-	B1364	10
96	B1405	18	B1392	574	B1411	6
96	B1419	125	B1434	953	B1418	28
96	B1428	36	B1438	32	B1443	17

-Sample not analyzed.

TABLE 3.2.12. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT LIVER FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Liver As (ng/g)	Animal Number	Liver As (ng/g)	Animal Number	Liver As (ng/g)
0					B1231	32
0					B1315	25
0					B1412	-
0					B1423	33
0					B1441	13
4	B1319	-	B1367	2,755		
4	B1394	-	B1373	3,899		
4	B1430	597	B1375	2,350		
4	B1437	927	B1389	2,240		
4	B1450	1,363	B1416	1,385		
12	B1374	624	B1316	3,962		
12	B1404	263	B1363	1,813		
12	B1422	176	B1395	3,285		
12	B1442	178	B1400	-		
12	B1444	585	B1449	2,479		
24	B1352	156	B1318	1,328		
24	B1358	103	B1332	1,830		
24	B1378	384	B1387	709		
24	B1420	455	B1421	645		
24	B1439	81	B1424	1,554		
48	B1312	136	B1205	599		
48	B1356	118	B1354	1,108		
48	B1379	183	B1362	991		
48	B1386	114	B1369	1,937		
48	B1440	245	B1397	1,333		
96	B1196	134	B1357	623	B1314	43
96	B1381	105	B1383	777	B1364	11
96	B1405	140	B1392	187	B1411	-
96	B1419	28	B1434	433	B1418	19
96	B1428	41	B1438	778	B1443	55

-Sample not analyzed.

TABLE 3.2.13. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT KIDNEY FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Kidney As (ng/g)	Animal Number	Kidney As (ng/g)	Animal Number	Kidney As (ng/g)
0					B1231	79
0					B1315	<20
0					B1412	34
0					B1423	52
0					B1441	23
4	B1319	3,316	B1367	2,857		
4	B1394	1,511	B1373	3,529		
4	B1430	4,533	B1375	2,305		
4	B1437	3,021	B1389	1,925		
4	B1450	1,544	B1416	2,597		
12	B1374	785	B1316	2,592		
12	B1404	1,139	B1363	1,423		
12	B1422	1,940	B1395	1,549		
12	B1442	807	B1400	1,699		
12	B1444	869	B1449	1,837		
24	B1352	350	B1318	883		
24	B1358	256	B1332	693		
24	B1378	530	B1387	1,446		
24	B1420	157	B1421	1,472		
24	B1439	379	B1424	1,456		
48	B1312	333	B1205	1,441		
48	B1356	134	B1354	1,004		
48	B1379	103	B1362	1,671		
48	B1386	122	B1369	1,601		
48	B1440	158	B1397	1,689		
96	B1196	138	B1357	969	B1314	<11
96	B1381	80	B1383	550	B1364	14
96	B1405	81	B1392	556	B1411	16
96	B1419	51	B1434	548	B1418	18
96	B1428	50	B1438	429	B1443	19

TABLE 3.2.14. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT TESTIS FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Testis As (ng/g)	Animal Number	Testis As (ng/g)	Animal Number	Testis As (ng/g)
0					B1231	14
0					B1315	11
0					B1412	16
0					B1423	13
0					B1441	28
4	B1319	401	B1367	327		
4	B1394	146	B1373	197		
4	B1430	229	B1375	115		
4	B1437	443	B1389	186		
4	B1450	254	B1416	193		
12	B1374	105	B1316	175		
12	B1404	124	B1363	151		
12	B1422	42	B1395	106		
12	B1442	153	B1400	71		
12	B1444	81	B1449	307		
24	B1352	92	B1318	156		
24	B1358	93	B1332	92		
24	B1378	161	B1387	198		
24	B1420	185	B1421	98		
24	B1439	97	B1424	296		
48	B1312	45	B1205	132		
48	B1356	48	B1354	138		
48	B1379	17	B1362	42		
48	B1386	50	B1369	155		
48	B1440	79	B1397	278		
96	B1196	13	B1357	392	B1314	13
96	B1381	59	B1383	148	B1364	<8
96	B1405	-	B1392	248	B1411	<9
96	B1419	19	B1434	61	B1418	17
96	B1428	37	B1438	160	B1443	<6

-Sample not analyzed.

TABLE 3.2.15. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT FAT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Fat As (ng/g)	Animal Number	Fat As (ng/g)	Animal Number	Fat As (ng/g)
0					B1231	<3
0					B1315	<3
0					B1412	6
0					B1423	<3
0					B1441	13
4	B1319	334	B1367	25		
4	B1394	<127	B1373	<4		
4	B1430	97	B1375	228		
4	B1437	116	B1389	60		
4	B1450	205	B1416	152		
12	B1374	118	B1316	58		
12	B1404	257	B1363	33		
12	B1422	-	B1395	67		
12	B1442	-	B1400	-		
12	B1444	-	B1449	-		
24	B1352	44	B1318	19		
24	B1358	20	B1332	16		
24	B1378	132	B1387	68		
24	B1420	18	B1421	59		
24	B1439	27	B1424	43		
48	B1312	<5	B1205	21		
48	B1356	23	B1354	21		
48	B1379	16	B1362	22		
48	B1386	<5	B1369	44		
48	B1440	-	B1397	49		
96	B1196	<6	B1357	23	B1314	<3
96	B1381	42	B1383	<3	B1364	<3
96	B1405	19	B1392	34	B1411	<3
96	B1419	13	B1434	10	B1418	8
96	B1428	4	B1438	-	B1442	<3

-Sample not analyzed.

TABLE 3.2.16. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT DOSE-SITE SKIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Dose Skin As (ng/g)	Animal Number	Dose Skin As (ng/g)	Animal Number	Dose Skin As (ng/g)
0					B1231	240
0					B1315	639
0					B1412	238
0					B1423	<308
0					B1441	306
4	B1319	18,839	B1367	11,413		
4	B1394	22,003	B1373	14,528		
4	B1430	17,634	B1375	5,436		
4	B1437	26,790	B1389	10,203		
4	B1450	37,020	B1416	20,219		
12	B1374	5,165	B1316	10,280		
12	B1404	8,956	B1363	6,130		
12	B1422	17,434	B1395	7,347		
12	B1442	15,207	B1400	10,452		
12	B1444	11,170	B1449	17,898		
24	B1352	4,821	B1318	10,163		
24	B1358	2,610	B1332	9,922		
24	B1378	12,899	B1387	6,391		
24	B1420	7,370	B1421	4,794		
24	B1439	6,701	B1424	2,322		
48	B1312	4,051	B1205	2,894		
48	B1356	8,910	B1354	5,285		
48	B1379	2,370	B1362	7,862		
48	B1386	4,286	B1369	2,802		
48	B1440	5,457	B1397	3,493		
96	B1196	5,133	B1357	5,339	B1314	631
96	B1381	2,945	B1383	4,948	B1364	639
96	B1405	3,220	B1392	4,627	B1411	109
96	B1419	16,767	B1434	2,268	B1418	37
96	B1428	8,147	B1438	3,504	B1443	199

TABLE 3.2.17. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT NORMAL SKIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Normal Skin As (ng/g)	Animal Number	Normal Skin As (ng/g)	Animal Number	Normal Skin As (ng/g)
0					B1231	30
0					B1315	42
0					B1412	40
0					B1423	37
0					B1441	593
4	B1319	719	B1367	707		
4	B1394	1,659	B1373	137		
4	B1430	401	B1375	-		
4	B1437	513	B1389	479		
4	B1450	588	B1416	1,536		
12	B1374	295	B1316	210		
12	B1404	671	B1363	614		
12	B1422	145	B1395	222		
12	B1442	175	B1400	312		
12	B1444	357	B1449	238		
24	B1352	161	B1318	141		
24	B1358	118	B1332	392		
24	B1378	310	B1387	442		
24	B1420	140	B1421	197		
24	B1439	206	B1424	139		
48	B1312	663	B1205	-		
48	B1356	110	B1354	296		
48	B1379	143	B1362	288		
48	B1386	40	B1369	1,861		
48	B1440	49	B1397	253		
96	B1196	99	B1357	114	B1314	-
96	B1381	106	B1383	435	B1364	18
96	B1405	148	B1392	108	B1411	22
96	B1419	991	B1434	124	B1418	21
96	B1428	56	B1438	94	B1443	11

-Sample not analyzed.

TABLE 3.2.18. GROUP MEAN (STANDARD DEVIATION) ARSENIC CONCENTRATION (ng/g) IN TISSUES AT VARYING TIMES AFTER L APPLICATION (L DOSE = 2.4 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Blood	L Alone	471	(205)	360	(99)	193	(21)	171	(40)	90	(18)
	L & BAL	477	(216)	137	(44)	69	(13)	47	(6)	41	(9)
	Vehicle Only	20	(10)							24	(7)
Brain	L Alone	165	(45)	156	(29)	175	(20)	194	(31)	206	(40)
	L & BAL	171	(35)	69	(27)	66	(24)	33	(16)	25	(5)
	Vehicle Only	6	(2)							6	(1)
Spinal Cord	L Alone	85	(16)	104	(28)	125	(74)	144	(58)	118	(17)
	L & BAL	220	(47)	80	(19)	49	(14)	39	(13)	21	(6)
	Vehicle Only	18	(9)							11	(4)
Lung	L Alone	3,806	(2,116)	2,347	(1,167)	920	(675)	1,311	(1,147)	564	(388)
	L & BAL	1,364	(1,000)	309	(96)	414	(179)	171	(176)	80	(64)
	Vehicle Only	28	(19)							14	(9)
Liver	L Alone	2,526	(915)	2,88	(937)	1,213	(521)	1,194	(493)	560	(252)
	L & BAL	962	(384)	365	(222)	236	(172)	159	(55)	90	(52)
	Vehicle Only	26	(9)							32	(20)
Testis	L Alone	204	(77)	162	(90)	168	(84)	149	(84)	202	(125)
	L & BAL	295	(124)	101	(42)	126	(44)	48	(22)	32	(21)
	Vehicle Only	16	(7)							11	(4)
Kidney	L Alone	2,643	(605)	1,820	(459)	1,190	(373)	1,481	(284)	610	(207)
	L & BAL	2,785	(1,280)	1,108	(486)	334	(140)	170	(93)	80	(36)
	Vehicle Only	42	(24)							16	(3)
Fat	L Alone	94	(94)	53	(18)	41	(23)	31	(14)	18	(14)
	L & BAL	176	(98)	188	(98)	48	(48)	12	(9)	17	(15)
	Vehicle Only	6	(4)							4	(2)

TABLE 3.2.18. (Continued)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Dose-Site Skin	L Alone	12,360	(5,476)	10,421	(4,577)	6,718	(3,364)	6,467	(4,441)	4,137	(1,249)
	L & BAL	24,457	(7,865)	11,586	(4,889)	6,880	(3,840)	5,015	(2,440)	7,242	(5,715)
	Vehicle Only	346	(167)							323	(291)
Normal Skin	L Alone	715	(596)	319	(170)	263	(144)	734	(652)	175	(146)
	L & BAL	776	(507)	329	(210)	187	(76)	201	(262)	280	(399)
	Vehicle Only	148	(249)							18	(5)

]Denotes no statistically significant difference between or among groups at alpha = 0.01 (two-sided); otherwise, group means are different from each other ($P<0.01$).

TABLE 3.2.19. WHOLE ORGAN BRAIN ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
0					B1231	<0.05
0					B1315	0.10
0					B1412	<0.05
0					B1423	<0.04
0					B1441	<0.04
4	B1319	2.04	B1367	1.24		
4	B1394	1.60	B1373	1.96		
4	B1430	1.41	B1375	1.30		
4	B1437	1.19	B1389	-		
4	B1450	-	B1416	1.07		
12	B1374	0.91	B1316	1.89		
12	B1404	0.89	B1363	1.20		
12	B1422	0.45	B1395	1.31		
12	B1442	0.31	B1400	1.17		
12	B1444	0.57	B1449	1.31		
24	B1352	0.62	B1318	1.43		
24	B1358	-	B1332	1.54		
24	B1378	0.43	B1387	1.53		
24	B1420	0.88	B1421	1.31		
24	B1439	0.52	B1424	1.76		
48	B1312	0.27	B1205	1.25		
48	B1356	0.28	B1354	1.91		
48	B1379	0.19	B1362	1.61		
48	B1386	0.20	B1369	1.49		
48	B1440	0.51	B1397	1.94		
96	B1196	0.16	B1357	2.34	B1314	<0.06
96	B1381	0.20	B1383	2.02	B1364	<0.04
96	B1405	0.29	B1392	1.56	B1411	<0.06
96	B1419	0.20	B1434	1.83	B1418	<0.04
96	B1428	0.23	B1438	1.46	B1443	<0.05

-Whole brain arsenic content not determined.

TABLE 3.2.20. WHOLE ORGAN LUNGS ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (µg)	Animal Number	As Content (µg)	Animal Number	As Content (µg)
0					B1231	0.32
0					B1315	0.53
0					B1412	0.18
0					B1423	1.61
0					B1441	0.48
4	B1319	4.73	B1367	44.65		
4	B1394	12.59	B1373	63.82		
4	B1430	22.07	B1375	4.81		
4	B1437	26.47	B1389	55.42		
4	B1450	20.44	B1416	42.40		
12	B1374	4.37	B1316	42.49		
12	B1404	4.06	B1363	21.60		
12	B1422	2.70	B1395	26.01		
12	B1442	3.67	B1400	24.06		
12	B1444	1.70	B1449	29.02		
24	B1352	6.16	B1318	15.75		
24	B1358	1.63	B1332	29.22		
24	B1378	3.11	B1387	18.68		
24	B1420	5.14	B1421	17.74		
24	B1439	3.89	B1424	13.26		
48	B1312	6.02	B1205	32.38		
48	B1356	2.17	B1354	11.03		
48	B1379	0.34	B1362	10.11		
48	B1386	3.49	B1369	17.21		
48	B1440	1.07	B1397	12.89		
96	B1196	1.46	B1357	13.88	B1314	0.09
96	B1381	1.58	B1383	-	B1364	0.24
96	B1405	0.41	B1392	7.64	B1411	0.21
96	B1419	1.42	B1434	9.64	B1418	0.84
96	B1428	0.48	B1438	0.74	B1443	0.26

-Whole lung arsenic content not determined.

TABLE 3.2.21. WHOLE ORGAN LIVER ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
0					B1231	3.14
0					B1315	2.34
0					B1412	-
0					B1423	4.48
0					B1441	1.28
4	B1319	-	B1367	281.23		
4	B1394	-	B1373	374.54		
4	B1430	36.43	B1375	238.20		
4	B1437	114.60	B1389	249.87		
4	B1450	145.95	B1416	174.44		
12	B1374	60.95	B1316	506.30		
12	B1404	34.11	B1363	220.41		
12	B1422	20.46	B1395	250.48		
12	B1442	12.44	B1400	-		
12	B1444	71.25	B1449	386.95		
24	B1352	19.06	B1318	171.22		
24	B1358	9.55	B1332	185.91		
24	B1378	33.15	B1387	81.28		
24	B1420	61.28	B1421	86.82		
24	B1439	7.61	B1424	163.93		
48	B1312	16.38	B1205	49.94		
48	B1356	14.80	B1354	113.15		
48	B1379	23.05	B1362	84.90		
48	B1386	10.73	B1369	160.36		
48	B1440	25.35	B1397	109.25		
96	B1196	16.88	B1357	54.05	B1314	6.72
96	B1381	11.32	B1383	63.95	B1364	1.37
96	B1405	22.79	B1392	-	B1411	-
96	B1419	4.04	B1434	35.86	B1418	2.33
96	B1428	4.04	B1438	71.46	B1443	6.85

-Whole liver arsenic content not determined.

TABLE 3.2.22. WHOLE ORGAN KIDNEYS ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
0					B1231	1.32
0					B1315	<0.34
0					B1412	0.53
0					B1423	0.83
0					B1441	0.39
4	B1319	59.36	B1367	35.54		
4	B1394	21.89	B1373	41.89		
4	B1430	57.48	B1375	35.13		
4	B1437	46.46	B1389	30.11		
4	B1450	24.94	B1416	36.72		
12	B1374	11.79	B1316	44.79		
12	B1404	27.50	B1363	23.32		
12	B1422	29.27	B1395	24.10		
12	B1442	11.25	B1400	28.71		
12	B1444	16.02	B1449	36.24		
24	B1352	6.18	B1318	23.36		
24	B1358	4.12	B1332	14.37		
24	B1378	7.16	B1387	28.40		
24	B1420	3.01	B1421	27.98		
24	B1439	6.20	B1424	25.41		
48	B1312	6.33	B1205	20.12		
48	B1356	2.27	B1354	18.55		
48	B1379	1.89	B1362	22.44		
48	B1386	2.11	B1369	24.43		
48	B1440	2.18	B1397	23.34		
96	B1196	3.00	B1357	19.73	B1314	<0.23
96	B1381	1.00	B1383	7.72	B1364	0.22
96	B1405	1.34	B1392	7.29	B1411	0.23
96	B1419	0.83	B1434	7.78	B1418	0.34
96	B1428	0.82	B1438	6.29	B1443	0.28

TABLE 3.2.23. WHOLE ORGAN TESTES ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II		III	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
0					B1231	0.03
0					B1315	0.04
0					B1412	0.02
0					B1423	0.02
0					B1441	0.03
4	B1319	1.29	B1367	0.30		
4	B1394	0.31	B1373	0.39		
4	B1430	0.42	B1375	0.19		
4	B1437	0.52	B1389	0.28		
4	B1450	0.54	B1416	0.23		
12	B1374	0.21	B1316	0.40		
12	B1404	0.20	B1363	0.11		
12	B1422	0.14	B1395	0.12		
12	B1442	0.11	B1400	0.16		
12	B1444	0.13	B1449	0.24		
24	B1352	0.16	B1318	0.28		
24	B1358	0.12	B1332	0.35		
24	B1378	0.17	B1387	0.30		
24	B1420	0.29	B1421	0.33		
24	B1439	0.17	B1424	0.27		
48	B1312	0.12	B1205	0.20		
48	B1356	0.06	B1354	0.41		
48	B1379	0.02	B1362	0.05		
48	B1386	0.06	B1369	0.39		
48	B1440	0.14	B1397	0.33		
96	B1196	0.03	B1357	0.25	B1314	0.04
96	B1381	0.04	B1383	0.20	B1364	<0.02
96	B1405	-	B1392	0.20	B1411	<0.02
96	B1419	0.03	B1434	0.08	B1418	0.03
96	B1428	0.03	B1438	0.26	B1443	<0.01

-Whole testes arsenic content not determined.

TABLE 3.2.24. DOSE-SITE SKIN ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
4	B1319	268.26	B1367	263.98
4	B1394	319.27	B1373	232.89
4	B1430	190.62	B1375	96.34
4	B1437	394.62	B1389	179.57
4	B1450	346.51	B1416	364.34
12	B1374	82.28	B1316	219.48
12	B1404	142.32	B1363	121.92
12	B1422	192.12	B1395	122.03
12	B1442	126.22	B1400	362.57
12	B1444	191.45	B1449	282.07
24	B1352	90.39	B1318	190.65
24	B1358	66.21	B1332	354.82
24	B1378	94.68	B1387	166.99
24	B1420	102.59	B1421	127.37
24	B1439	56.15	B1424	88.61
48	B1312	86.03	B1205	59.32
48	B1356	78.85	B1354	74.68
48	B1379	44.07	B1362	190.49
48	B1386	88.08	B1369	61.20
48	B1440	92.34	B1397	265.01
96	B1196	79.82	B1357	54.62
96	B1381	25.48	B1383	86.24
96	B1405	38.48	B1392	83.88
96	B1419	160.29	B1434	63.64
96	B1428	113.24	B1438	97.72

TABLE 3.2.25. GROUP MEAN (STANDARD DEVIATION) WHOLE ORGAN ARSENIC CONTENT (μg) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 2.4 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	1.39]	(0.39)	1.38	(0.29)	1.52	(0.17)	1.64	(0.29)	1.84	(0.36)
	L & BAL	1.56]	(0.36)	0.63	(0.27)	0.61	(0.19)	0.29	(0.13)	0.22]	(0.05)
	Vehicle Only	0.06	(0.02)							0.05]	(0.01)
Lungs	L Alone	42.2]	(22.6)	28.6	(8.2)	18.9	(6.1)	16.7	(9.2)	8.0	(5.5)
	L & BAL	17.3]	(8.6)	3.3	(1.1)	4.0	(1.8)	2.6	(2.2)	1.1]	(0.6)
	Vehicle Only	0.6	(0.6)							0.3]	(0.3)
Liver	L Alone	263.7]	(73.1)	341.0	(131.9)	137.8	(49.8)	103.5	(40.5)	56.3	(15.4)
	L & BAL	99.0]	(56.4)	39.8	(25.5)	26.1	(22.1)	18.1	(6.0)	11.8	(8.2)
	Vehicle Only	2.8	(1.4)							4.3	(2.9)
Kidneys	L Alone	35.9]	(4.2)	31.4	(9.1)	23.9	(5.7)	21.8	(2.4)	9.8	(5.6)
	L & BAL	42.0]	(17.7)	19.2	(8.6)	5.3	(1.7)	3.0	(1.9)	1.4]	(0.9)
	Vehicle Only	0.7	(0.4)							0.3]	(0.1)
Testes	L Alone	0.28	(0.08)	0.20]	(0.12)	0.30	(0.03)	0.28	(0.15)	0.20]	(0.07)
	L & BAL	0.62	(0.39)	0.16]	(0.04)	0.18]	(0.06)	0.08]	(0.05)	0.03]	(<0.01)
	Vehicle Only	0.03	(0.01)							0.02]	(0.01)
Dose-Site Skin	L Alone	227.2]	(99.8)	221.6]	(104.1)	185.7	(102.2)	130.1	(93.1)	77.2]	(17.6)
	L & BAL	303.9]	(78.1)	146.9]	(46.5)	82.0	(19.8)	77.9	(19.5)	83.5]	(55.2)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

TABLE 3.2.26. WHOLE ORGAN BRAIN ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	I L & BAL		II L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	0.083	B1367	0.067
4	B1394	0.072	B1373	0.100
4	B1430	0.070	B1375	0.064
4	B1437	0.059	B1389	-
4	B1450	-	B1416	0.053
12	B1374	0.043	B1316	0.070
12	B1404	0.034	B1363	0.065
12	B1422	0.023	B1395	0.073
12	B1442	0.018	B1400	0.053
12	B1444	0.027	B1449	0.063
24	B1352	0.028	B1318	0.060
24	B1358	-	B1332	0.060
24	B1378	0.023	B1387	0.070
24	B1420	0.039	B1421	0.050
24	B1439	0.024	B1424	0.088
48	B1312	0.010	B1205	0.055
48	B1356	0.013	B1354	0.085
48	B1379	0.009	B1362	0.086
48	B1386	0.011	B1369	0.071
48	B1440	0.027	B1397	0.100
96	B1196	0.007	B1357	0.128
96	B1381	0.011	B1383	0.103
96	B1405	0.014	B1392	0.087
96	B1419	0.009	B1434	0.096
96	B1428	0.012	B1438	0.083

-Percent brain arsenic content not determined.

TABLE 3.2.27. WHOLE ORGAN LUNG ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	0.19	B1367	2.41
4	B1394	0.57	B1373	3.25
4	B1430	1.10	B1375	0.24
4	B1437	1.31	B1389	2.70
4	B1450	0.93	B1416	2.10
12	B1374	0.21	B1316	1.57
12	B1404	0.15	B1363	1.16
12	B1422	0.14	B1395	1.45
12	B1442	0.22	B1400	1.09
12	B1444	0.08	B1449	1.38
24	B1352	0.28	B1318	0.66
24	B1358	0.08	B1332	1.14
24	B1378	0.16	B1387	0.85
24	B1420	0.23	B1421	0.68
24	B1439	0.18	B1424	0.66
48	B1312	0.23	B1205	1.42
48	B1356	0.10	B1354	0.49
48	B1379	0.02	B1362	0.54
48	B1386	0.20	B1369	0.83
48	B1440	0.06	B1397	0.66
96	B1196	0.06	B1357	0.76
96	B1381	0.09	B1383	-
96	B1405	0.02	B1392	0.42
96	B1419	0.07	B1434	0.50
96	B1428	0.02	B1438	0.04

-Percent lung arsenic content not determined.

TABLE 3.2.28. WHOLE ORGAN LIVER ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	-	B1367	15.19
4	B1394	-	B1373	19.10
4	B1430	1.81	B1375	11.79
4	B1437	5.67	B1389	12.16
4	B1450	6.66	B1416	8.62
12	B1374	2.89	B1316	18.72
12	B1404	1.30	B1363	11.83
12	B1422	1.02	B1395	13.93
12	B1442	0.73	B1400	-
12	B1444	3.41	B1449	18.42
24	B1352	0.87	B1318	7.19
24	B1358	0.47	B1332	7.25
24	B1378	1.76	B1387	3.69
24	B1420	2.71	B1421	3.33
24	B1439	0.36	B1424	8.17
48	B1312	0.63	B1205	2.19
48	B1356	0.71	B1354	5.01
48	B1379	1.06	B1362	4.53
48	B1386	0.60	B1369	7.69
48	B1440	1.31	B1397	5.62
96	B1196	0.71	B1357	2.97
96	B1381	0.62	B1383	3.28
96	B1405	1.11	B1392	-
96	B1419	0.19	B1434	1.88
96	B1428	0.21	B1438	4.05

-Percent liver arsenic content not determined.

TABLE 3.2.29. WHOLE ORGAN KIDNEYS ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	2.41	B1367	1.92
4	B1394	0.99	B1373	2.14
4	B1430	2.86	B1375	1.74
4	B1437	2.30	B1389	1.47
4	B1450	1.14	B1416	1.81
12	B1374	0.56	B1316	1.66
12	B1404	1.05	B1363	1.25
12	B1422	1.47	B1395	1.34
12	B1442	0.66	B1400	1.30
12	B1444	0.77	B1449	1.73
24	B1352	0.28	B1318	0.98
24	B1358	0.20	B1332	0.56
24	B1378	0.38	B1387	1.29
24	B1420	0.13	B1421	1.07
24	B1439	0.29	B1424	1.27
48	B1312	0.24	B1205	0.88
48	B1356	0.11	B1354	0.82
48	B1379	0.09	B1362	1.20
48	B1386	0.12	B1369	1.17
48	B1440	0.11	B1397	1.20
96	B1196	0.13	B1357	1.08
96	B1381	0.05	B1383	0.40
96	B1405	0.07	B1392	0.40
96	B1419	0.04	B1434	0.41
96	B1428	0.04	B1438	0.36

TABLE 3.2.30. WHOLE ORGAN TESTES ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group / Treatment	I L & BAL		II L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	0.0523	B1367	0.0164
4	B1394	0.0141	B1373	0.0197
4	B1430	0.0207	B1375	0.0093
4	B1437	0.0259	B1389	0.0138
4	B1450	0.0246	B1416	0.0113
12	B1374	0.0098	B1316	0.0149
12	B1404	0.0074	B1363	0.0057
12	B1422	0.0073	B1395	0.0067
12	B1442	0.0062	B1400	0.0071
12	B1444	0.0064	B1449	0.0113
24	B1352	0.0073	B1318	0.0117
24	B1358	0.0062	B1332	0.0135
24	B1378	0.0091	B1387	0.0137
24	B1420	0.0130	B1421	0.0126
24	B1439	0.0078	B1424	0.0133
48	B1312	0.0045	B1205	0.0087
48	B1356	0.0027	B1354	0.0180
48	B1379	0.0010	B1362	0.0028
48	B1386	0.0035	B1369	0.0185
48	B1440	0.0073	B1397	0.0172
96	B1196	0.0013	B1357	0.0136
96	B1381	0.0022	B1383	0.0102
96	B1405	-	B1392	0.0110
96	B1419	0.0017	B1434	0.0040
96	B1428	0.0016	B1438	0.0150

-Percent testes arsenic content not determined.

TABLE 3.2.31. WHOLE ORGAN DOSE SITE SKIN ARSENIC CONTENT AS A PERCENT OF THE TOTAL DUSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 2.4 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B1319	10.89	B1367	14.26
4	B1394	14.43	B1373	11.87
4	B1430	9.48	B1375	4.72
4	B1437	19.51	B1389	8.74
4	B1450	15.80	B1415	18.00
12	B1374	3.90	B1316	8.11
12	B1404	5.41	B1363	6.54
12	B1422	9.62	B1395	6.79
12	B1442	7.44	B1400	16.40
12	B1444	9.10	B1449	13.43
24	B1352	4.13	B1318	8.01
24	B1358	3.29	B1332	13.84
24	B1378	5.03	B1387	7.58
24	B1420	4.53	B1421	4.88
24	B1439	2.65	B1424	4.40
48	B1312	3.33	B1205	2.60
48	B1356	3.80	B1354	3.31
48	B1379	2.02	B1362	10.16
48	B1386	4.92	B1369	2.94
48	B1440	4.76	B1397	13.63
96	B1196	3.34	B1357	3.00
96	B1381	1.39	B1383	4.42
96	B1405	1.87	B1392	4.66
96	B1419	7.67	B1434	3.33
96	B1428	5.85	B1438	5.54

Note: Dose-site skin weights for the vehicle control group are not presented, since lesions were not well defined at the dose site in these animals.

TABLE 3.2.32. GROUP MEAN (STANDARD DEVIATION) WHOLE ORGAN ARSENIC CONTENT AS A PORTION OF THE TOTAL DOSE (%) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 2.4 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	0.071]	(0.020)	0.065	(0.008)	0.066	(0.019)	0.079	(0.017)	0.099	(0.018)
	L & BAL	0.071]	(0.010)	0.029	(0.010	0.029	(0.007)	0.014	(0.007)	0.011	(0.003)
Lungs	L Alone	2.14	(1.15)	1.33	(0.20)	0.80	(0.21)	0.79	(0.38)	0.43]	(0.30)
	L & BAL	0.82	(0.44)	0.16	(0.06)	0.19	(0.07)	0.12	(0.09)	0.05]	(0.03)
Liver	L Alone	13.37	(3.96)	15.73	(3.40)	5.93	(2.24)	5.01	(1.99)	3.04	(0.90)
	L & BAL	4.71	(2.56)	1.87	(1.20)	1.23	(0.99)	0.86	(0.31)	0.57	(0.38)
Kidneys	L Alone	1.81]	(0.25)	1.45	(0.22)	1.03	(0.29)	1.05	(0.19)	0.53	(0.31)
	L & BAL	1.94]	(0.83)	0.90	(0.36)	0.26	(0.09)	0.13	(0.06)	0.07	(0.04)
Testes	L Alone	0.014	(0.004)	0.009]	(0.004)	0.013]	(<0.001)	0.013	(0.007)	0.011]	(0.004)
	L & BAL	0.028	(0.015)	0.007]	(0.001)	0.009]	(0.003)	0.004	(0.002)	0.002]	(<0.001)
Dose-Site Skin	L Alone	11.52]	(5.09)	10.25]	(4.42)	7.74	(3.76)	6.53	(5.06)	4.19]	(1.03)
	L & BAL	14.02]	(4.00)	7.11]	(2.44)	3.93	(0.96)	3.77	(1.18)	4.02]	(2.68)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

TABLE 3.2.33. RABBIT BRAIN WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	IV L & BAL		V VI L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Brain Weight (g)	Animal Number	Brain Weight (g)	Animal Number	Brain Weight (g)
0					B4885	7.68
0					B4916	8.90
0					B4930	9.03
0					B4934	7.91
0					B4936	8.32
4	B4691	8.95	B4897	9.15		
4	B4725	8.90	B4900	8.29		
4	B4913	8.35	B4911	9.70		
4	B4927	8.17	B4960	8.55		
4	B4957	7.47	B4984	8.48		
12	B4714	9.24	B4891	8.68		
12	B4920	9.32	B4893	7.78		
12	B4926	8.69	B4906	8.09		
12	B4940	8.29	B4925	8.23		
12	B4968	8.67	B4974	8.09		
24	B4731	8.28	B4908	8.26		
24	B4914	8.55	B4923	7.90		
24	B4931	9.05	B4941	8.60		
24	B4948	8.78	B4976	7.25		
24	B4970	9.07	B4979	8.67		
48	B4944	8.52	B4722	9.73		
48	B4955	8.21	B4902	8.66		
48	B4959	7.82	B4915	8.05		
48	B4963	7.52	B4953	8.44		
48	B4989	7.86	B4969	9.10		
96	B4708	8.83	B4898	8.22	B4686	8.51
96	B4713	7.86	B4939	7.93	B4924	7.94
96	B4895	9.03	B4949	8.59	B4967	9.42
96	B4938	8.68	B4956	8.79	B4980	9.42
96	B4958	8.10	B4981	9.19	B4990	7.90

TABLE 3.2.34. RABBIT LUNGS WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Lungs Weight (g)	Animal Number	Lungs Weight (g)	Animal Number	Lungs Weight (g)
0					B4885	9.37
0					B4916	26.97
0					B4930	10.89
0					B4934	9.71
0					B4936	14.36
4	B4691	11.62	B4897	11.97		
4	B4725	10.50	B4900	12.39		
4	B4913	20.46	B4911	24.10		
4	B4927	8.27	B4960	25.19		
4	B4957	11.34	B4984	20.20		
12	B4714	10.40	B4891	9.18		
12	B4920	28.19	B4893	21.85		
12	B4926	9.34	B4906	23.39		
12	B4940	15.71	B4925	9.88		
12	B4968	10.72	B4974	27.03		
24	B4731	21.70	B4908	28.70		
24	B4914	9.27	B4923	20.89		
24	B4931	15.67	B4941	25.78		
24	B4948	10.77	B4976	11.43		
24	B4970	8.77	B4979	14.21		
48	B4944	8.28	B4722	10.67		
48	B4955	9.95	B4902	10.79		
48	B4959	11.89	B4915	13.90		
48	B4963	11.77	B4953	32.31		
48	B4989	8.70	B4969	11.60		
96	B4708	16.88	B4898	10.45	B4686	14.51
96	B4713	18.91	B4939	22.12	B4924	9.60
96	B4895	17.66	B4949	16.42	B4967	16.23
96	B4938	10.34	B4956	24.57	B4980	32.90
96	B4958	21.70	B4981	31.73	B4990	40.29

TABLE 3.2.35. RABBIT LIVER WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Liver Weight (g)	Animal Number	Liver Weight (g)	Animal Number	Liver Weight (g)
0					B4885	89.77
0					B4916	113.00
0					B4930	155.32
0					B4934	99.60
0					B4936	188.75
4	B4691	113.03	B4897	94.89		
4	B4725	87.42	B4900	73.45		
4	B4913	102.25	B4911	98.83		
4	B4927	70.22	B4960	98.35		
4	B4957	81.83	B4984	109.09		
12	B4714	94.71	B4891	78.91		
12	B4920	115.92	B4893	92.37		
12	B4926	81.86	B4906	118.39		
12	B4940	106.34	B4925	73.22		
12	B4968	102.04	B4974	96.15		
24	B4731	126.72	B4908	105.51		
24	B4914	124.75	B4923	77.89		
24	B4931	98.36	B4941	130.93		
24	B4948	154.97	B4976	90.98		
24	B4970	75.59	B4979	70.36		
48	B4944	85.58	B4722	108.88		
48	B4955	117.87	B4902	97.10		
48	B4959	97.44	B4915	106.66		
48	B4963	86.50	B4953	98.78		
48	B4989	83.95	B4969	114.94		
96	B4708	111.25	B4898	94.38	B4686	103.45
96	B4713	111.05	B4939	89.07	B4924	113.61
96	B4895	91.96	B4949	97.99	B4967	95.23
96	B4938	116.61	B4956	74.72	B4980	95.18
96	B4958	116.95	B4981	98.85	B4990	85.02

TABLE 3.2.36. RABBIT KIDNEYS WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Kidneys Weight (g)	Animal Number	Kidneys Weight (g)	Animal Number	Kidneys Weight (g)
0					B4885	12.45
0					B4916	16.68
0					B4930	17.36
0					B4934	15.55
0					B4936	16.90
4	B4691	18.34	B4897	14.60		
4	B4725	15.71	B4900	13.17		
4	B4913	16.20	B4911	14.90		
4	B4927	12.49	B4960	15.64		
4	B4957	16.47	B4984	13.89		
12	B4714	17.48	B4891	16.25		
12	B4920	16.02	B4893	12.48		
12	B4926	15.31	B4906	14.05		
12	B4940	17.05	B4925	15.70		
12	B4968	14.31	B4974	11.82		
24	B4731	20.02	B4908	18.23		
24	B4914	16.57	B4923	13.53		
24	B4931	13.47	B4941	16.29		
24	B4948	15.78	B4976	15.54		
24	B4970	15.27	B4979	15.48		
48	B4944	13.58	B4722	20.52		
48	B4955	14.36	B4902	20.94		
48	B4959	14.29	B4915	22.13		
48	B4963	14.78	B4953	18.37		
48	B4989	14.26	B4969	16.23		
96	B4708	17.34	B4898	18.92	B4686	26.56
96	B4713	13.78	B4939	19.90	B4924	14.33
96	B4895	13.89	B4949	13.89	B4967	14.04
96	B4938	18.73	B4956	16.01	B4980	15.03
96	B4958	12.76	B4981	19.12	B4990	12.51

TABLE 3.2.37. RABBIT TESTES WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Testes Weight (g)	Animal Number	Testes Weight (g)	Animal Number	Testes Weight (g)
0					B4885	1.43
0					B4916	2.57
0					B4930	2.27
0					B4934	1.77
0					B4936	2.73
4	B4691	2.07	B4897	2.47		
4	B4725	3.12	B4900	1.07		
4	B4913	2.39	B4911	2.72		
4	B4927	1.39	B4960	1.61		
4	B4957	1.05	B4984	1.99		
12	B4714	2.34	B4891	1.42		
12	B4920	3.09	B4893	1.86		
12	B4926	1.51	B4906	1.60		
12	B4940	2.32	B4925	0.85		
12	B4968	1.46	B4974	1.54		
24	B4731	3.25	B4908	1.86		
24	B4914	2.55	B4923	0.99		
24	B4931	3.32	B4941	2.50		
24	B4948	2.19	B4976	1.42		
24	B4970	2.33	B4979	0.80		
48	B4944	1.14	B4722	2.11		
48	B4955	2.27	B4902	1.29		
48	B4959	1.08	B4915	1.13		
48	B4963	1.31	B4953	2.32		
48	B4989	1.48	B4969	2.60		
96	B4708	2.55	B4898	1.46	B4686	4.79
96	B4713	2.95	B4939	3.35	B4924	1.58
96	B4895	2.10	B4949	2.33	B4967	2.95
96	B4938	3.60	B4956	2.07	B4980	2.58
96	B4958	3.49	B4981	1.97	B4990	1.23

TABLE 3.2.38. DOSE-SITE SKIN WEIGHT (g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hrs) post-dosing)	Animal Number	Dose-Site Skin Wt (g)	Animal Number	Dose-Site Skin Wt (g)
4	B4691	25.25	B4897	16.63
4	B4725	11.19	B4900	9.55
4	B4913	6.10	B4911	12.45
4	B4927	8.38	B4960	18.30
4	B4957	7.71	B4984	14.59
12	B4714	20.99	B4891	12.83
12	B4920	25.30	B4893	27.84
12	B4926	14.13	B4906	22.40
12	B4940	13.68	B4925	17.90
12	B4968	15.97	B4974	29.50
24	B4731	12.44	B4908	42.11
24	B4914	15.80	B4923	22.79
24	B4931	16.85	B4941	36.13
24	B4948	18.98	B4976	20.75
24	B4970	11.57	B4979	17.35
48	B4944	8.81	B4722	32.04
48	B4955	17.46	B4902	31.26
48	B4959	13.95	B4915	17.99
48	B4963	17.52	B4953	19.83
48	B4989	10.52	B4969	33.68
96	B4708	21.39	B4898	15.50
96	B4713	21.02	B4939	21.34
96	B4895	12.86	B4949	34.93
96	B4938	21.22	B4956	25.89
96	B4958	15.67	B4981	16.85

Note: Dose-site skin weights for the vehicle control group are not presented, since lesions were not well defined at the dose site in these animals.

TABLE 3.2.39. GROUP MEAN (STANDARD DEVIATION) ORGAN WEIGHTS (g) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 3.5 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	8.8]	(0.6)	8.2]	(0.3)	8.1]	(0.6)	8.8]	(0.7)	8.3]	(0.3)
	L & BAL	8.4]	(0.6)	8.8]	(0.4)	8.8]	(0.3)	8.0]	(0.4)	8.5]	(0.5)
	Vehicle Only	8.4]	(0.6)							8.6]	(0.8)
Lungs	L Alone	18.8]	(6.3)	18.3]	(8.2)	20.2]	(7.4)	15.9]	(9.3)	21.1]	(8.1)
	L & BAL	12.4]	(4.7)	14.9]	(7.8)	13.2]	(5.5)	10.1]	(1.7)	17.1]	(4.2)
	Vehicle Only	14.3]	(7.4)							22.7]	(13.2)
Liver	L Alone	94.9]	(13.1)	91.8]	(17.6)	95.1]	(24.1)	105.3]	(7.4)	91.0]	(9.9)
	L & BAL	91.0]	(16.9)	100.2]	(12.8)	116.1]	(30.2)	94.3]	(14.2)	109.6]	(10.2)
	Vehicle Only	129.3]	(41.6)							98.5]	(10.7)
Kidneys	L Alone	14.4]	(1.0)	14.1]	(1.9)	15.8]	(1.7)	19.6	(2.3)	17.6	(2.5)
	L & BAL	15.8]	(2.1)	16.0]	(1.3)	16.2]	(2.4)	14.3]	(0.4)	15.3]	(2.6)
	Vehicle Only	15.8]	(2.0)							16.5]	(5.7)
Testes	L Alone	2.0]	(0.7)	1.5]	(0.4)	1.5]	(0.7)	1.9]	(0.7)	2.2]	(0.7)
	L & BAL	2.0]	(0.8)	2.1]	(0.7)	2.7]	(0.5)	1.5]	(0.5)	2.9]	(0.6)
	Vehicle Only	2.2]	(0.6)							2.6]	(1.4)
Dose-Site Skin	L Alone	14.3]	(3.5)	22.1	(6.9)	27.8	(10.7)	27.0	(7.4)	22.9]	(7.9)
	L & BAL	11.7]	(7.8)	18.0	(5.0)	15.1	(3.1)	13.7	(4.0)	18.4]	(3.9)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

TABLE 3.2.40. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT BLOOD FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	IV L & BAL		V L Alone		VI Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Blood As (ng/g)	Animal Number	Blood As (ng/g)	Animal Number	Blood As (ng/g)
0					B4885	<6
0					B4916	8
0					B4930	8
0					B4934	8
0					B4936	20
4	B4691	569	B4897	362		
4	B4725	632	B4900	515		
4	B4913	315	B4911	488		
4	B4927	324	B4960	-		
4	B4957	335	B4984	387		
12	B4714	313	B4891	354		
12	B4920	62	B4893	294		
12	B4926	76	B4906	311		
12	B4940	66	B4925	470		
12	B4968	128	B4974	377		
24	B4731	28	B4908	240		
24	B4914	61	B4923	114		
24	B4931	46	B4941	170		
24	B4948	35	B4976	283		
24	B4970	55	B4979	159		
48	B4944	31	B4722	109		
48	B4955	32	B4902	230		
48	B4959	24	B4915	197		
48	B4963	35	B4953	136		
48	B4989	39	B4969	106		
96	B4708	23	B4898	107	B4686	9
96	B4713	28	B4939	100	B4924	6
96	B4895	17	B4949	90	B4967	<6
96	B4938	19	B4956	87	B4981	7
96	B4958	24	B4981	133	B4990	6

-Sample not analyzed.

TABLE 3.2.41. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT BRAIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Brain As (ng/g)	Animal Number	Brain As (ng/g)	Animal Number	Brain As (ng/g)
0					B4885	10
0					B4916	<7
0					B4930	9
0					B4934	8
0					B4936	-
4	B4691	120	B4897	133		
4	B4725	263	B4900	198		
4	B4913	155	B4911	149		
4	B4927	340	B4960	226		
4	B4957	248	B4984	129		
12	B4714	61	B4891	270		
12	B4920	57	B4893	258		
12	B4926	58	B4906	192		
12	B4940	66	B4925	239		
12	B4968	67	B4974	250		
24	B4731	59	B4908	257		
24	B4914	84	B4923	232		
24	B4931	107	B4941	224		
24	B4948	52	B4976	269		
24	B4970	89	B4979	392		
48	B4944	63	B4722	238		
48	B4955	51	B4902	374		
48	B4959	54	B4915	319		
48	B4963	53	B4953	259		
48	B4989	57	B4969	187		
96	B4708	34	B4898	357	B4686	10
96	B4713	31	B4939	257	B4924	27
96	B4895	50	B4949	274	B4967	27
96	B4938	30	B4956	313	B4980	9
96	B4958	38	B4981	343	B4990	9

-Sample not analyzed.

TABLE 3.2.42. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT SPINAL CORD FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Spinal Cord As (ng/g)	Animal Number	Spinal Cord As (ng/g)	Animal Number	Spinal Cord As (ng/g)
0					B4885	<13
0					B4916	<10
0					B4930	<10
0					B4934	<12
0					B4936	<30
4	B4691	220	B4897	50		
4	B4725	601	B4900	-		
4	B4913	284	B4911	143		
4	B4927	369	B4960	170		
4	B4957	475	B4984	145		
12	B4714	99	B4891	155		
12	B4920	35	B4893	113		
12	B4926	92	B4906	117		
12	B4940	69	B4925	240		
12	B4968	101	B4974	167		
24	B4731	-	B4908	230		
24	B4914	92	B4923	201		
24	B4931	52	B4941	244		
24	B4948	63	B4976	-		
24	B4970	-	B4979	283		
48	B4944	36	B4722	127		
48	B4955	34	B4902	305		
48	B4959	34	B4915	268		
48	B4963	35	B4953	158		
48	B4989	48	B4969	114		
96	B4708	<18	B4898	258	B4686	<10
96	B4713	41	B4939	-	B4924	<29
96	B4895	32	B4949	132	B4967	<10
96	B4938	61	B4956	354	B4980	-
96	B4958	15	B4981	352	B4990	<17

-Sample not analyzed.

TABLE 3.2.43. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT LUNG FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	IV L & BAL		V L Alone		VI Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Lung As (ng/g)	Animal Number	Lung As (ng/g)	Animal Number	Lung As (ng/g)
0					B4885	16
0					B4916	15
0					B4930	34
0					B4934	29
0					B4936	15
4	B4691	997	B4897	5,505		
4	B4725	1,480	B4900	6,091		
4	B4913	1,339	B4911	3,895		
4	B4927	1,242	B4960	4,197		
4	B4957	2,056	B4984	3,400		
12	B4714	428	B4891	4,136		
12	B4920	179	B4893	2,453		
12	B4926	397	B4906	1,745		
12	B4940	227	B4925	4,352		
12	B4968	368	B4974	2,557		
24	B4731	272	B4908	1,230		
24	B4914	604	B4923	852		
24	B4931	751	B4941	2,218		
24	B4948	303	B4976	1,544		
24	B4970	442	B4979	1,636		
48	B4944	308	B4722	1,874		
48	B4955	434	B4902	1,969		
48	B4959	303	B4915	1,723		
48	B4963	361	B4953	803		
48	B4989	486	B4969	1,260		
96	B4708	183	B4898	1,339	B4686	18
96	B4713	176	B4939	583	B4924	17
96	B4895	127	B4949	498	B4967	13
96	B4938	248	B4956	852	B4980	10
96	B4958	215	B4981	704	B4990	17

TABLE 3.2.44. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT LIVER FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Liver As (ng/g)	Animal Number	Liver As (ng/g)	Animal Number	Liver As (ng/g)
0					B4885	20
0					B4916	16
0					B4930	16
0					B4934	18
0					B4936	6
4	B4691	1,553	B4897	2,681		
4	B4725	4,524	B4900	4,498		
4	B4913	837	B4911	3,434		
4	B4927	1,384	B4960	7,259		
4	B4957	1,786	B4984	2,829		
12	B4714	399	B4891	6,497		
12	B4920	370	B4893	6,485		
12	B4926	214	B4906	4,398		
12	B4940	388	B4925	4,893		
12	B4968	548	B4974	7,176		
24	B4731	355	B4908	3,105		
24	B4914	705	B4923	4,015		
24	B4931	406	B4941	2,744		
24	B4948	200	B4976	3,725		
24	B4970	417	B4979	4,472		
48	B4944	232	B4722	2,794		
48	B4955	279	B4902	2,700		
48	B4959	248	B4915	1,952		
48	B4963	223	B4953	2,231		
48	B4989	-	B4969	586		
96	B4708	111	B4898	685	B4686	13
96	B4713	218	B4939	1,337	B4924	13
96	B4895	115	B4949	907	B4967	10
96	B4938	124	B4956	1,292	B4980	<12
96	B4958	148	B4981	962	B4990	15

-Sample not analyzed.

TABLE 3.2.45. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT KIDNEY FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Kidney As (ng/g)	Animal Number	Kidney As (ng/g)	Animal Number	Kidney As (ng/g)
0					B4885	28
0					B4916	20
0					B4930	17
0					B4934	24
0					B4936	25
4	B4691	1,733	B4897	5,758		
4	B4725	4,526	B4900	4,808		
4	B4913	3,954	B4911	6,286		
4	B4927	2,624	B4960	6,950		
4	B4957	5,870	B4984	4,059		
12	B4714	944	B4891	4,147		
12	B4920	684	B4893	2,752		
12	B4926	923	B4906	4,536		
12	B4940	945	B4925	6,065		
12	B4968	1,090	B4974	5,836		
24	B4731	346	B4908	2,128		
24	B4914	962	B4923	1,257		
24	B4931	867	B4941	2,717		
24	B4948	399	B4976	2,873		
24	B4970	327	B4979	2,583		
48	B4944	263	B4722	1,758		
48	B4955	322	B4902	2,484		
48	B4959	245	B4915	1,348		
48	B4963	50*	B4953	1,525		
48	B4989	311	B4969	904		
96	B4708	213	B4898	963	B4686	28
96	B4713	220	B4939	987	B4924	17
96	B4895	289	B4949	959	B4967	20
96	B4938	314	B4956	1,313	B4980	6
96	B4958	250	B4981	1,609	B4990	21

*Outlier as determined by two-sided outlier test at alpha = 0.0026 (±3 standard deviations).

TABLE 3.2.46. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT TESTIS FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Testis As (ng/g)	Animal Number	Testis As (ng/g)	Animal Number	Testis As (ng/g)
0					B4885	<10
0					B4916	38
0					B4930	<6
0					B4934	15
0					B4936	6
4	B4691	182	B4897	194		
4	B4725	507	B4900	341		
4	B4913	250	B4911	249		
4	B4927	509	B4960	303		
4	B4957	561	B4984	230		
12	B4714	111	B4891	370		
12	B4920	49	B4893	377		
12	B4926	98	B4906	199		
12	B4940	67	B4925	518		
12	B4968	-	B4974	374		
24	B4731	75	B4908	558		
24	B4914	165	B4923	356		
24	B4931	108	B4941	254		
24	B4948	93	B4976	645		
24	B4970	72	B4979	669		
48	B4944	32	B4722	197		
48	B4955	25	B4902	445		
48	B4959	44	B4915	350		
48	B4963	89	B4953	323		
48	B4989	77	B4969	201		
96	B4708	42	B4898	391	B4686	6
96	B4713	61	B4939	230	B4924	13
96	B4895	30	B4949	196	B4967	29
96	B4938	15	B4956	290	B4980	34
96	B4958	27	B4981	254	B4990	22

-Sample not analyzed.

TABLE 3.2.47. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT ABDOMINAL FAT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Fat As (ng/g)	Animal Number	Fat As (ng/g)	Animal Number	Fat As (ng/g)
0					B4885	9
0					B4916	10
0					B4930	-
0					B4934	7
0					B4936	12
4	B4691	725	B4897	275		
4	B4725	3,592	B4900	345		
4	B4913	1,753	B4911	420		
4	B4927	2,148	B4960	410		
4	B4957	1,953	B4984	178		
12	B4714	521	B4891	319		
12	B4920	330	B4893	217		
12	B4926	186	B4906	154		
12	B4940	232	B4925	264		
12	B4968	191	B4974	209		
24	B4731	71	B4908	169		
24	B4914	781	B4923	31		
24	B4931	77	B4941	52		
24	B4948	21	B4976	282		
24	B4970	442	B4979	109		
48	B4944	26	B4722	132		
48	B4955	31	B4902	321		
48	B4959	25	B4915	91		
48	B4963	93	B4953	248		
48	B4989	64	B4969	105		
96	B4708	15	B4898	135	B4686	45
96	B4713	14	B4939	57	B4924	15
96	B4895	15	B4949	129	B4967	30
96	B4938	16	B4956	116	B4980	9
96	B4958	12	B4981	180	B4990	38

-Sample not analyzed.

TABLE 3.2.48. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT DOSE-SITE SKIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Dose Skin As (ng/g)	Animal Number	Dose Skin As (ng/g)	Animal Number	Dose Skin As (ng/g)
0					B4885	675
0					B4916	11
0					B4930	54
0					B4934	28
0					B4936	348
4	B4691	12,771	B4897	19,783		
4	B4725	52,433	B4900	36,664		
4	B4913	89,469	B4911	51,945		
4	B4927	48,740	B4960	35,946		
4	B4957	62,314	B4984	-		
12	B4714	19,050	B4891	28,012		
12	B4920	7,928	B4893	9,857		
12	B4926	22,557	B4906	7,514		
12	B4940	14,667	B4925	13,054		
12	B4968	5,936	B4974	-		
24	B4731	26,814	B4908	9,995		
24	B4914	20,111	B4923	12,873		
24	B4931	15,036	B4941	10,084		
24	B4948	19,949	B4976	13,823		
24	B4970	7,543	B4979	26,764		
48	B4944	11,841	B4722	-		
48	B4955	9,117	B4902	11,170		
48	B4959	9,207	B4915	-		
48	B4963	16,963	B4953	12,570		
48	B4989	9,618	B4969	7,188		
96	B4708	8,335	B4898	7,020	B4686	42
96	B4713	28,621	B4939	14,495	B4924	366
96	B4895	7,142	B4949	8,765	B4967	48
96	B4938	1,196	B4956	4,241	B4980	199
96	B4958	10,425	B4981	8,423	B4990	64

-Sample not analyzed.

TABLE 3.2.49. ARSENIC CONCENTRATIONS (ng/g) IN RABBIT NORMAL SKIN FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	Normal Skin As (ng/g)	Animal Number	Normal Skin As (ng/g)	Animal Number	Normal Skin As (ng/g)
0					B4885	11
0					B4916	7
0					B4930	<11
0					B4934	4
0					B4936	8
4	B4691	517	B4897	258		
4	B4725	832	B4900	-		
4	B4913	481	B4911	275		
4	B4927	536	B4960	307		
4	B4957	312	B4984	382		
12	B4714	544	B4891	299		
12	B4920	491	B4893	241		
12	B4926	287	B4906	341		
12	B4940	280	B4925	311		
12	B4968	161	B4974	289		
24	B4731	491	B4908	255		
24	B4914	267	B4923	573		
24	B4931	373	B4941	288		
24	B4948	228	B4976	304		
24	B4970	165	B4979	-		
48	B4944	42	B4722	264		
48	B4955	130	B4902	356		
48	B4959	-	B4915	371		
48	B4963	34	B4953	356		
48	B4989	50	B4969	256		
96	B4708	200	B4898	193	B4686	15
96	B4713	291	B4939	320	B4924	<4
96	B4895	98	B4949	142	B4967	<14
96	B4938	259	B4956	350	B4980	<9
96	B4958	436	B4981	400	B4990	5

-Sample not analyzed.

TABLE 3.2.50. GROUP MEAN (STANDARD DEVIATION) ARSENIC CONCENTRATION (ng/g) IN TISSUES AT VARYING TIMES AFTER L APPLICATION (L DOSE = 3.5 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Blood	L Alone	438	(75)	361	(69)	193	(68)	156	(55)	103	(18)
	L & BAL	435	(153)	129	(106)	45	(14)	32	(6)	22	(4)
	Vehicle Only	10	(6)							7	(1)
Brain	L Alone	167	(43)	242	(30)	275	(68)	275	(73)	309	(43)
	L & BAL	225	(88)	62	(5)	78	(23)	56	(5)	37	(8)
	Vehicle Only	9	(1)							16	(10)
Spinal Cord	L Alone	127	(53)	158	(51)	240	(34)	194	(87)	274	(105)
	L & BAL	390	(152)	79	(28)	69	(21)	37	(6)	33	(19)
	Vehicle Only	15	(8)							17	(9)
Lung	L Alone	4,618	(1,134)	3,049	(1,138)	1,496	(507)	1,526	(487)	795	(332)
	L & BAL	1,423	(395)	320	(110)	474	(203)	378	(80)	190	(45)
	Vehicle Only	22	(9)							15	(3)
Liver	L Alone	4,140	(1,884)	5,890	(1,183)	3,612	(694)	2,053	(889)	1,037	(275)
	L & BAL	2,017	(1,445)	384	(119)	417	(183)	246	(25)	143	(44)
	Vehicle Only	15	(5)							13	(2)
Testis	L Alone	263	(59)	368	(113)	496	(183)	303	(105)	272	(75)
	L & BAL	402	(173)	81	(28)	103	(38)	53	(28)	35	(17)
	Vehicle Only	15	(13)							21	(11)
Kidney	L Alone	5,572	(1,153)	4,667	(1,349)	2,312	(652)	1,604	(583)	1,166	(289)
	L & BAL	3,741	(1,618)	917	(146)	580	(308)	285	(37)	257	(44)
	Vehicle Only	23	(4)							18	(8)
Fat	L Alone	326	(101)	233	(62)	129	(101)	179	(100)	123	(44)
	L & BAL	2,034	(1,029)	292	(140)	278	(328)	48	(30)	14	(2)
	Vehicle Only	10	(2)							27	(15)

TABLE 3.2.50. (Continued)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Dose-Site Skin	L Alone	36,084]	(13,136)	14,609]	(9,219)	14,708]	(6,948)	10,309]	(2,793)	8,589]	(3,752)
	L & BAL	53,145]	(27,629)	14,028]	(7,090)	17,891]	(7,142)	11,349]	(3,329)	11,144]	(10,354)
	Vehicle Only	223	(288)							144	(140)
Normal Skin	L Alone	306]	(55)	296]	(37)	355]	(147)	321	(56)	281]	(109)
	L & BAL	536]	(188)	353]	(160)	305]	(129)	64	(44)	256]	(124)
	Vehicle Only	8	(3)							9	(5)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

TABLE 3.2.51. WHOLE ORGAN BRAIN ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (µg)	Animal Number	As Content (µg)	Animal Number	As Content (µg)
0					B4885	0.08
0					B4916	<0.06
0					B4930	0.08
0					B4934	0.06
0					B4936	-
4	B4691	1.07	B4897	1.22		
4	B4725	2.34	B4900	1.64		
4	B4913	1.29	B4911	1.45		
4	B4927	2.78	B4960	1.93		
4	B4957	1.85	B4984	1.09		
12	B4714	0.56	B4891	2.34		
12	B4920	0.53	B4893	2.01		
12	B4926	0.50	B4906	1.55		
12	B4940	0.55	B4925	1.97		
12	B4968	0.58	B4974	2.02		
24	B4731	0.49	B4908	2.12		
24	B4914	0.72	B4923	1.83		
24	B4931	0.97	B4941	1.93		
24	B4948	0.46	B4976	1.95		
24	B4970	0.81	B4979	3.40		
48	B4944	0.54	B4722	2.32		
48	B4955	0.42	B4902	3.24		
48	B4959	0.42	B4915	2.57		
48	B4963	0.40	B4953	2.19		
48	B4989	0.45	B4969	1.70		
96	B4708	0.30	B4898	2.93	B4686	0.09
96	B4713	0.24	B4939	2.04	B4924	0.21
96	B4895	0.45	B4949	2.35	B4967	0.25
96	B4938	0.26	B4956	2.75	B4980	0.08
96	B4958	0.31	B4981	2.81	B4990	0.07

-Whole brain arsenic content not determined.

TABLE 3.2.52. WHOLE ORGAN LUNGS ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (µg)	Animal Number	As Content (µg)	Animal Number	As Content (µg)
0					B4885	0.15
0					B4916	0.40
0					B4930	0.37
0					B4934	0.28
0					B4936	0.22
4	B4691	11.59	B4897	65.89		
4	B4725	15.54	B4900	75.47		
4	B4913	27.40	B4911	93.87		
4	B4927	10.27	B4960	105.72		
4	B4957	23.32	B4984	68.68		
12	B4714	4.45	B4891	37.97		
12	B4920	5.05	B4893	53.60		
12	B4926	3.71	B4906	40.82		
12	B4940	3.57	B4925	43.00		
12	B4968	3.94	B4974	69.12		
24	B4731	5.90	B4908	35.30		
24	B4914	5.60	B4923	17.80		
24	B4931	11.77	B4941	57.18		
24	B4948	3.26	B4976	17.65		
24	B4970	3.88	B4979	23.25		
48	B4944	2.55	B4722	20.00		
48	B4955	4.32	B4902	21.25		
48	B4959	3.60	B4915	23.95		
48	B4963	4.25	B4953	25.94		
48	B4989	4.23	B4969	14.62		
96	B4708	3.09	B4898	13.99	B4686	0.26
96	B4713	3.33	B4939	12.90	B4924	0.16
96	B4895	2.24	B4949	8.18	B4967	0.21
96	B4938	2.56	B4956	20.93	B4980	0.33
96	B4958	4.67	B4981	22.34	B4990	0.68

TABLE 3.2.53. WHOLE ORGAN LIVER ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (µg)	Animal Number	As Content (µg)	Animal Number	As Content (µg)
0					B4885	1.80
0					B4916	1.81
0					B4930	2.49
0					B4934	1.79
0					B4936	1.13
4	B4691	175.54	B4897	254.40		
4	B4725	395.49	B4900	330.38		
4	B4913	85.58	B4911	339.38		
4	B4927	97.18	B4960	713.92		
4	B4957	146.15	B4984	308.62		
12	B4714	37.79	B4891	512.68		
12	B4920	42.89	B4893	599.02		
12	B4926	17.52	B4906	520.68		
12	B4940	41.26	B4925	358.27		
12	B4968	55.92	B4974	689.97		
24	B4731	44.99	B4908	327.61		
24	B4914	87.95	B4923	312.73		
24	B4931	39.93	B4941	359.27		
24	B4948	30.99	B4976	338.90		
24	B4970	31.52	B4979	314.65		
48	B4944	19.85	B4722	304.21		
48	B4955	32.89	B4902	262.17		
48	B4959	24.17	B4915	208.20		
48	B4963	19.29	B4953	220.38		
48	B4989	-	B4969	67.35		
96	B4708	12.35	B4898	64.65	B4686	1.34
96	B4713	24.21	B4939	119.09	B4924	1.48
96	B4895	10.58	B4949	88.88	B4967	0.95
96	B4938	14.46	B4956	96.54	B4980	<1.14
96	B4958	17.31	B4981	95.09	B4990	1.28

-Whole liver arsenic content not determined.

TABLE 3.2.54. WHOLE ORGAN KIDNEYS ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (μg)	Animal Number	As Content (μg)	Animal Number	As Content (μg)
0					B4885	0.35
0					B4916	0.33
0					B4930	0.30
0					B4934	0.37
0					B4936	0.42
4	B4691	31.78	B4897	84.07		
4	B4725	71.10	B4900	63.32		
4	B4913	64.05	B4911	93.66		
4	B4927	32.77	B4960	108.70		
4	B4957	96.68	B4984	56.38		
12	B4714	16.50	B4891	67.39		
12	B4920	10.96	B4893	34.34		
12	B4926	14.13	B4906	63.73		
12	B4940	16.11	B4925	95.22		
12	B4968	15.60	B4974	68.98		
24	B4731	6.93	B4908	38.79		
24	B4914	15.94	B4923	17.01		
24	B4931	11.68	B4941	44.26		
24	B4948	6.30	B4976	44.65		
24	B4970	4.99	B4979	40.01		
48	B4944	3.57	B4722	36.07		
48	B4955	4.62	B4902	52.01		
48	B4959	3.50	B4915	29.83		
48	B4963	-	B4953	28.01		
48	B4989	4.43	B4969	14.67		
96	B4708	3.69	B4898	18.22	B4686	0.74
96	B4713	3.03	B4939	19.64	B4924	0.24
96	B4895	4.01	B4949	13.32	B4967	0.28
96	B4938	5.88	B4956	21.02	B4980	0.09
96	B4958	3.19	B4981	30.76	B4990	0.26

-Whole kidney arsenic content not determined.

TABLE 3.2.55. WHOLE ORGAN TESTES ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	IV		V		VI	
Treatment	L & BAL		L Alone		Vehicle Control	
Nominal Sacrifice Time (Hours post-dosing)	Animal Number	As Content (µg)	Animal Number	As Content (µg)	Animal Number	As Content (µg)
0					B4885	<0.01
0					B4916	0.10
0					B4930	<0.01
0					B4934	0.03
0					B4936	0.02
4	B4691	0.38	B4897	0.48		
4	B4725	1.58	B4900	0.36		
4	B4913	0.60	B4911	0.68		
4	B4927	0.71	B4960	0.49		
4	B4957	0.59	B4984	0.46		
12	B4714	0.26	B4891	0.53		
12	B4920	0.15	B4893	0.70		
12	B4926	0.15	B4906	0.32		
12	B4940	0.16	B4925	0.44		
12	B4968	-	B4974	0.58		
24	B4731	0.24	B4908	1.04		
24	B4914	0.42	B4923	0.35		
24	B4931	0.36	B4941	0.64		
24	B4948	0.20	B4976	0.92		
24	B4970	0.17	B4979	0.54		
48	B4944	0.04	B4722	0.42		
48	B4955	0.06	B4902	0.57		
48	B4959	0.05	B4915	0.40		
48	B4963	0.12	B4953	0.75		
48	B4989	0.11	B4969	0.52		
96	B4708	0.11	B4898	0.57	B4686	0.03
96	B4713	0.18	B4939	0.77	B4924	0.02
96	B4895	0.06	B4949	0.46	B4967	0.09
96	B4938	0.05	B4956	0.60	B4980	0.09
96	B4958	0.09	B4981	0.50	B4990	0.03

-Whole testes arsenic content not determined.

TABLE 3.2.56. DOSE-SITE SKIN ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Nominal Sacrifice Time (Hrs) post-dosing)	Group Treatment	I L & BAL		II L Alone	
		Animal Number	As Content (μg)	Animal Number	As Content (μg)
4		B4691	322.47	B4897	328.99
4		B4725	586.73	B4900	350.14
4		B4913	545.76	B4911	646.72
4		B4927	408.44	B4960	657.81
4		B4957	480.44	B4984	-
12		B4714	399.86	B4891	359.39
12		B4920	200.57	B4893	274.42
12		B4926	318.73	B4906	168.32
12		B4940	200.64	B4925	233.66
12		B4968	94.80	B4974	-
24		B4731	333.57	B4908	420.89
24		B4914	317.75	B4923	293.37
24		B4931	253.36	B4941	364.33
24		B4948	378.62	B4976	286.84
24		B4970	87.27	B4979	464.35
48		B4944	104.32	B4722	-
48		B4955	159.19	B4902	349.18
48		B4959	128.44	B4915	-
48		B4963	297.19	B4953	249.26
48		B4989	101.18	B4969	242.07
96		B4708	178.30	B4898	108.81
96		B4713	601.60	B4939	309.33
96		B4895	91.85	B4949	306.15
96		B4938	25.38	B4956	109.79
96		B4958	163.36	B4981	141.93

-Percent dose-site skin arsenic content not determined.

TABLE 3.2.57. GROUP MEAN (STANDARD DEVIATION) WHOLE ORGAN ARSENIC CONTENT (µg) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 3.5 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	1.47]	(0.34)	1.98	(0.28)	2.25	(0.65)	2.40	(0.56)	2.58	(0.37)
	L & BAL	1.87]	(0.71)	0.55	(0.03)	0.69	(0.22)	0.44	(0.05)	0.31	(0.08)
	Vehicle Only	0.07	(0.01)							0.14	(0.09)
Lungs	L Alone	81.9	(17.2)	48.9	(12.8)	30.2	(16.7)	21.2	(4.3)	15.7	(5.9)
	L & BAL	17.6	(7.5)	4.1	(0.6)	6.1	(3.4)	3.8	(0.8)	3.2	(0.9)
	Vehicle Only	0.3	(0.1)							0.3	(0.2)
Liver	L Alone	389.3	(184.4)	536.1	(122.5)	330.6	(19.2)	212.5	(89.5)	92.9	(19.5)
	L & BAL	180.0	(125.9)	39.1	(13.9)	47.1	(23.6)	24.1	(6.3)	15.8	(5.3)
	Vehicle Only	1.8	(0.5)							1.2	(0.2)
Kidneys	L Alone	81.2]	(21.5)	65.9	(21.6)	36.9	(11.4)	32.1	(13.6)	20.6	(6.4)
	L & BAL	59.3]	(27.5)	14.7	(2.3)	9.2	(4.6)	4.0	(0.6)	4.0	(1.1)
	Vehicle Only	0.4								0.3	(0.3)
Testes	L Alone	0.49]	(0.11)	0.51	(0.14)	0.70	(0.28)	0.53	(0.14)	0.58	(0.12)
	L & BAL	0.77]	(0.47)	0.18	(0.05)	0.28]	(0.11)	0.07]	(0.04)	0.10]	(0.05)
	Vehicle Only	0.03	(0.04)							0.05]	(0.03)
Dose-Site Skin	L Alone	496]	(181)	259]	(80)	366]	(78)	280]	(60)	195]	(104)
	L & BAL	469]	(106)	243]	(118)	274]	(114)	158]	(81)	212]	(226)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

TABLE 3.2.58. WHOLE ORGAN BRAIN ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group	I		II	
Treatment	L & BAL		L Alone	
Nominal Sacrifice Time (Hrs) post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B4691	0.028	B4897	0.035
4	B4725	0.065	B4900	0.058
4	B4913	0.040	B4911	0.041
4	B4927	0.094	B4960	0.055
4	B4957	0.058	B4984	0.033
12	B4714	0.016	B4891	0.086
12	B4920	0.016	B4893	0.062
12	B4926	0.016	B4906	0.049
12	B4940	0.017	B4925	0.073
12	B4968	0.018	B4974	0.065
24	B4731	0.014	B4908	0.061
24	B4914	0.021	B4923	0.063
24	B4931	0.028	B4941	0.051
24	B4948	0.012	B4976	0.062
24	B4970	0.026	B4979	0.118
48	B4944	0.018	B4722	0.057
48	B4955	0.013	B4902	0.104
48	B4959	0.014	B4915	0.077
48	B4963	0.013	B4953	0.061
48	B4989	0.016	B4969	0.048
96	B4708	0.008	B4898	0.081
96	B4713	0.007	B4939	0.058
96	B4895	0.015	B4949	0.072
96	B4938	0.008	B4956	0.086
96	B4958	0.010	B4981	0.083

TABLE 3.2.59. WHOLE ORGAN LUNG ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Nominal Sacrifice Time (Hrs) post-dosing)	Group Treatment	I L & BAL		II L Alone	
		Animal Number	As Content (%)	Animal Number	As Content (%)
4		B4691	0.31	B4897	1.92
4		B4725	0.43	B4900	2.65
4		B4913	0.85	B4911	2.68
4		B4927	0.35	B4960	2.99
4		B4957	0.73	B4984	2.06
12		B4714	0.13	B4891	1.40
12		B4920	0.15	B4893	1.65
12		B4926	0.12	B4906	1.29
12		B4940	0.11	B4925	1.60
12		B4968	0.13	B4974	2.21
24		B4731	0.16	B4908	1.02
24		B4914	0.16	B4923	0.61
24		B4931	0.34	B4941	1.53
24		B4948	0.09	B4976	0.56
24		B4970	0.13	B4979	0.81
48		B4944	0.09	B4722	0.49
48		B4955	0.13	B4902	0.68
48		B4959	0.12	B4915	0.72
48		B4963	0.14	B4953	0.72
48		B4989	0.15	B4969	0.41
96		B4708	0.09	B4898	0.39
96		B4713	0.10	B4939	0.37
96		B4895	0.07	B4949	0.25
96		B4938	0.08	B4956	0.65
96		B4958	0.15	B4981	0.66

TABLE 3.2.60. WHOLE ORGAN LIVER ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	I L & BAL		II L Alone	
Nominal Sacrifice Time (Hrs) post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B4691	4.65	B4897	7.41
4	B4725	11.05	B4900	11.59
4	B4913	2.64	B4911	9.69
4	B4927	3.27	B4960	20.21
4	B4957	4.59	B4984	9.24
12	B4714	1.09	B4891	18.85
12	B4920	1.28	B4893	18.45
12	B4926	0.55	B4906	16.47
12	B4940	1.28	B4925	13.36
12	B4968	1.78	B4974	22.08
24	B4731	1.25	B4908	9.45
24	B4914	2.51	B4923	10.73
24	B4931	1.17	B4941	9.60
24	B4948	0.84	B4976	10.76
24	B4970	1.02	B4979	10.94
48	B4944	0.68	B4722	7.49
48	B4955	1.00	B4902	8.38
48	B4959	0.82	B4915	6.26
48	B4963	0.63	B4953	6.15
48	B4989	-	B4969	1.89
96	B4708	0.35	B4898	1.79
96	B4713	0.71	B4939	3.39
96	B4895	0.35	B4949	2.73
96	B4938	0.43	B4956	3.01
96	B4958	0.54	B4981	2.81

-Percent liver arsenic content not determined.

TABLE 3.2.61. WHOLE ORGAN KIDNEYS ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Group Treatment	I L & BAL		II L Alone	
Nominal Sacrifice Time (Hrs) post-dosing)	Animal Number	As Content (%)	Animal Number	As Content (%)
4	B4691	0.84	B4897	2.45
4	B4725	1.99	B4900	2.22
4	B4913	1.98	B4911	2.67
4	B4927	1.10	B4960	3.08
4	B4957	3.04	B4984	1.69
12	B4714	0.48	B4891	2.48
12	B4920	0.33	B4893	1.06
12	B4926	0.44	B4906	2.02
12	B4940	0.50	B4925	3.55
12	B4968	0.50	B4974	2.21
24	B4731	0.19	B4908	1.12
24	B4914	0.46	B4923	0.58
24	B4931	0.34	B4941	1.18
24	B4948	0.17	B4976	1.42
24	B4970	0.16	B4979	1.39
48	B4944	0.12	B4722	0.89
48	B4955	0.14	B4902	1.66
48	B4959	0.12	B4915	0.90
48	B4963	-	B4953	0.78
48	B4989	0.16	B4969	0.41
96	B4708	0.10	B4898	0.50
96	B4713	0.09	B4939	0.56
96	B4895	0.13	B4949	0.41
96	B4938	0.18	B4956	0.66
96	B4958	0.10	B4981	0.91

-Percent kidneys arsenic content not determined.

TABLE 3.2.62. WHOLE ORGAN TESTES ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Nominal Sacrifice Time (Hrs) post-dosing)	Group Treatment	I L & BAL		II L Alone	
		Animal Number	As Content (%)	Animal Number	As Content (%)
4		B4691	0.0100	B4897	0.0140
4		B4725	0.0442	B4900	0.0128
4		B4913	0.0185	B4911	0.0193
4		B4927	0.0238	B4960	0.0138
4		B4957	0.0185	B4984	0.0137
12		B4714	0.0075	B4891	0.0193
12		B4920	0.0045	B4893	0.0216
12		B4926	0.0046	B4906	0.0101
12		B4940	0.0048	B4925	0.0164
12		B4968	-	B4974	0.0184
24		B4731	0.0068	B4908	0.0299
24		B4914	0.0120	B4923	0.0121
24		B4931	0.0105	B4941	0.0170
24		B4948	0.0055	B4976	0.0291
24		B4970	0.0054	B4979	0.0186
48		B4944	0.0013	B4722	0.0102
48		B4955	0.0017	B4902	0.0184
48		B4959	0.0016	B4915	0.0119
48		B4963	0.0038	B4953	0.0209
48		B4989	0.0040	B4969	0.0147
96		B4708	0.0030	B4898	0.0158
96		B4713	0.0052	B4939	0.0220
96		B4895	0.0021	B4949	0.0141
96		B4938	0.0016	B4956	0.0187
96		B4958	0.0030	B4981	0.0148

-Percent testes arsenic content not determined.

TABLE 3.2.63. DOSE-SITE SKIN ARSENIC CONTENT AS A PERCENT OF THE TOTAL DOSE FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 3.5 mg/kg OF L WITH AND WITHOUT BAL THERAPY

Nominal Sacrifice Time (Hrs) post-dosing)	Group Treatment	I L & BAL		II L Alone	
		Animal Number	As Content (%)	Animal Number	As Content (%)
4		B4691	8.55	B4897	9.58
4		B4725	16.40	B4900	12.28
4		B4913	16.86	B4911	18.47
4		B4927	13.75	B4960	18.62
4		B4957	15.08	B4984	-
12		B4714	11.58	B4891	13.21
12		B4920	6.00	B4893	8.45
12		B4926	9.95	B4906	5.32
12		B4940	6.20	B4925	8.72
12		B4968	3.02	B4974	-
24		B4731	9.30	B4908	12.14
24		B4914	9.08	B4923	10.06
24		B4931	7.39	B4941	9.74
24		B4948	10.22	B4976	9.11
24		B4970	2.82	B4979	16.15
48		B4944	3.59	B4722	-
48		B4955	4.84	B4902	11.17
48		B4959	4.36	B4915	-
48		B4963	9.70	B4953	6.96
48		B4989	3.56	B4969	6.81
96		B4708	5.04	B4898	3.01
96		B4713	17.54	B4939	8.82
96		B4895	3.06	B4949	9.42
96		B4938	0.76	B4956	3.42
96		B4958	5.12	B4981	4.19

-Percent dose-site skin arsenic content not determined.

TABLE 3.2.64. GROUP MEAN (STANDARD DEVIATION) WHOLE ORGAN ARSENIC CONTENT AS A PORTION OF THE TOTAL DOSE (%) AT VARIOUS TIMES AFTER L APPLICATION (L DOSE = 3.5 mg/kg)

Tissue		Time Post L Dose in hours									
		4		12		24		48		96	
Brain	L Alone	0.044]	(0.011)	0.06/	(0.014)	0.071	(0.027)	0.069	(0.022)	0.076	(0.011)
	L & BAL	0.057]	(0.025)	0.017	(0.001)	0.020	(0.007)	0.015	(0.002)	0.010	(0.003)
Lungs	L Alone	2.46	(0.45)	1.63	(0.36)	0.91	(0.39)	0.61	(0.14)	0.46	(0.18)
	L & BAL	0.53	(0.24)	0.13	(0.02)	0.18	(0.10)	0.13	(0.02)	0.10	(0.03)
Liver	L Alone	11.63	(5.02)	17.84	(3.21)	10.30	(0.71)	6.03	(2.49)	2.75	(0.59)
	L & BAL	5.24	(3.36)	1.20	(0.44)	1.36	(0.67)	0.78	(0.17)	0.48	(0.15)
Kidneys	L Alone	2.42]	(0.52)	2.26	(0.90)	1.14	(0.34)	0.93	(0.46)	0.61]	(0.19)
	L & BAL	1.79]	(0.87)	0.45	(0.07)	0.26	(0.13)	0.13	(0.02)	0.12]	(0.04)
Testes	L Alone	0.015]	(0.003)	0.017	(0.004)	0.021	(0.008)	0.015	(0.004)	0.017	(0.003)
	L & BAL	0.0023]	(0.013)	0.005	(0.001)	0.008	(0.003)	0.002	(0.001)	0.003	(0.001)
Dose-Site Skin	L Alone	14.74]	(4.53)	8.93]	(3.25)	11.44]	(2.87)	8.31]	(2.47)	5.77]	(3.09)
	L & BAL	14.13]	(3.35)	7.35]	(3.41)	7.76]	(2.95)	5.21]	(2.57)	6.30]	(6.53)

]Denotes no statistically significant difference between or among groups at alpha = 0.01; otherwise, group means are different from each other (P<0.01).

APPENDIX D

Figures

LEGEND FOR FIGURES 3.2.1 THROUGH 3.2.16

Group	I	II	III
L Dose	2.4 mg/kg	2.4 mg/kg	none
Therapy	BAL	none	none
Data Values	□	△	c
Regression Curves	————	- - - - -	— - — -

LEGEND FOR FIGURES 3.2.17 THROUGH 3.2.32

Group	IV	V	VI
L Dose	3.5 mg/kg	3.5 mg/kg	none
Therapy	BAL	none	none
Data Values	□	△	c
Regression Curves	————	- - - - - - - - - -	————

LEGEND FOR FIGURES 3.2.33 THROUGH 3.2.48

Group	I	II	IV	V	III & VI
L Dose	2.4 mg/kg	2.4 mg/kg	3.5 mg/kg	3.5 mg/kg	none
Therapy	BAL	none	BAL	none	none
Regression Curves	----------	————	—·—·—	————	————

FIGURE 3.1.1. PROBIT ANALYSIS COMPOSITE PLOT FOR DILUTE LEWISITE ADMINISTERED SUBCUTANEOUSLY IN RABBITS

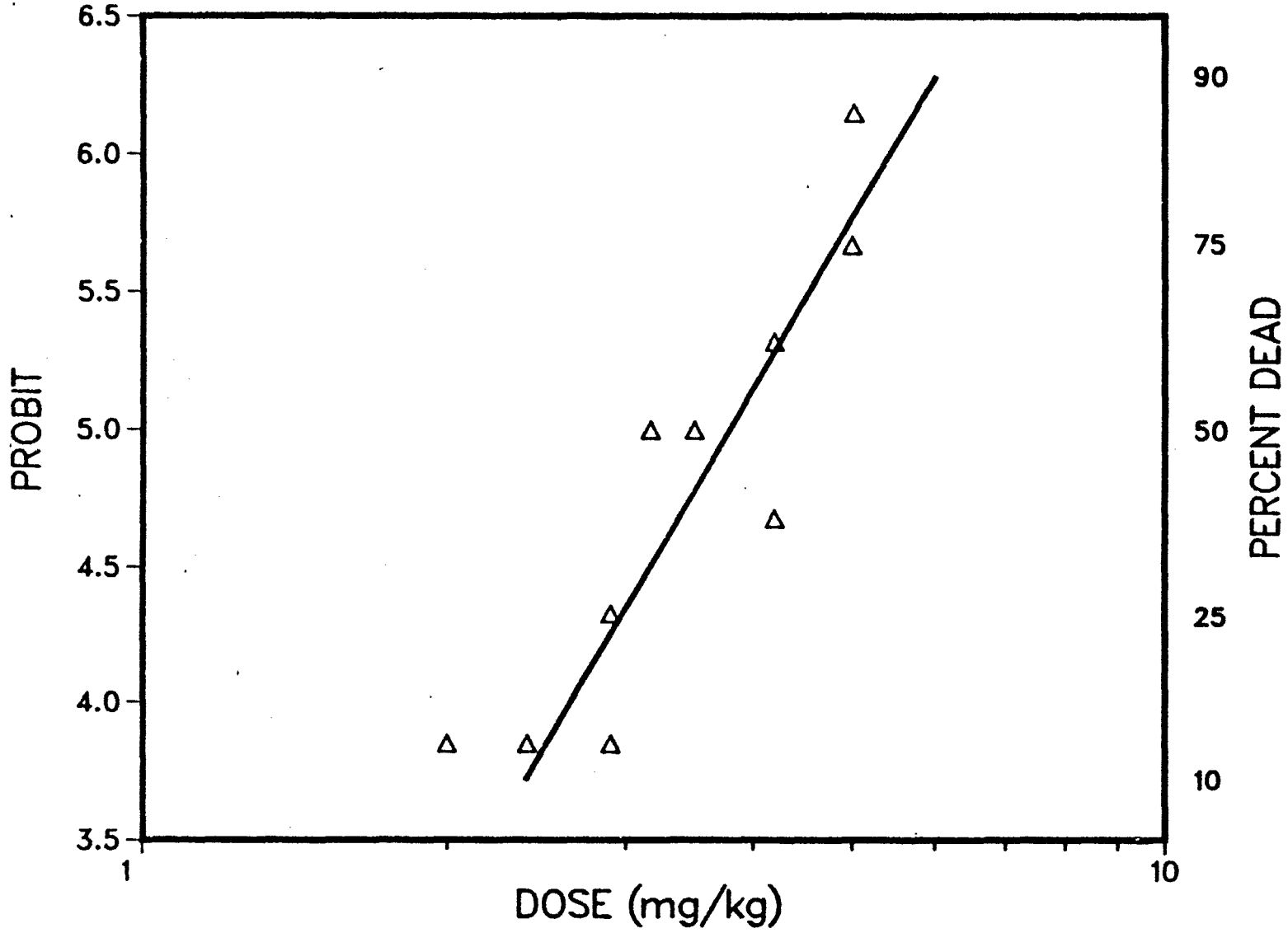


FIGURE 3.1.2. PROBIT ANALYSIS COMPOSITE PLOT FOR DILUTE BRITISH ANTI-LEWISITE ADMINISTERED IN QUADRUPLICATE INJECTIONS INTRAMUSCULARLY IN RABBITS

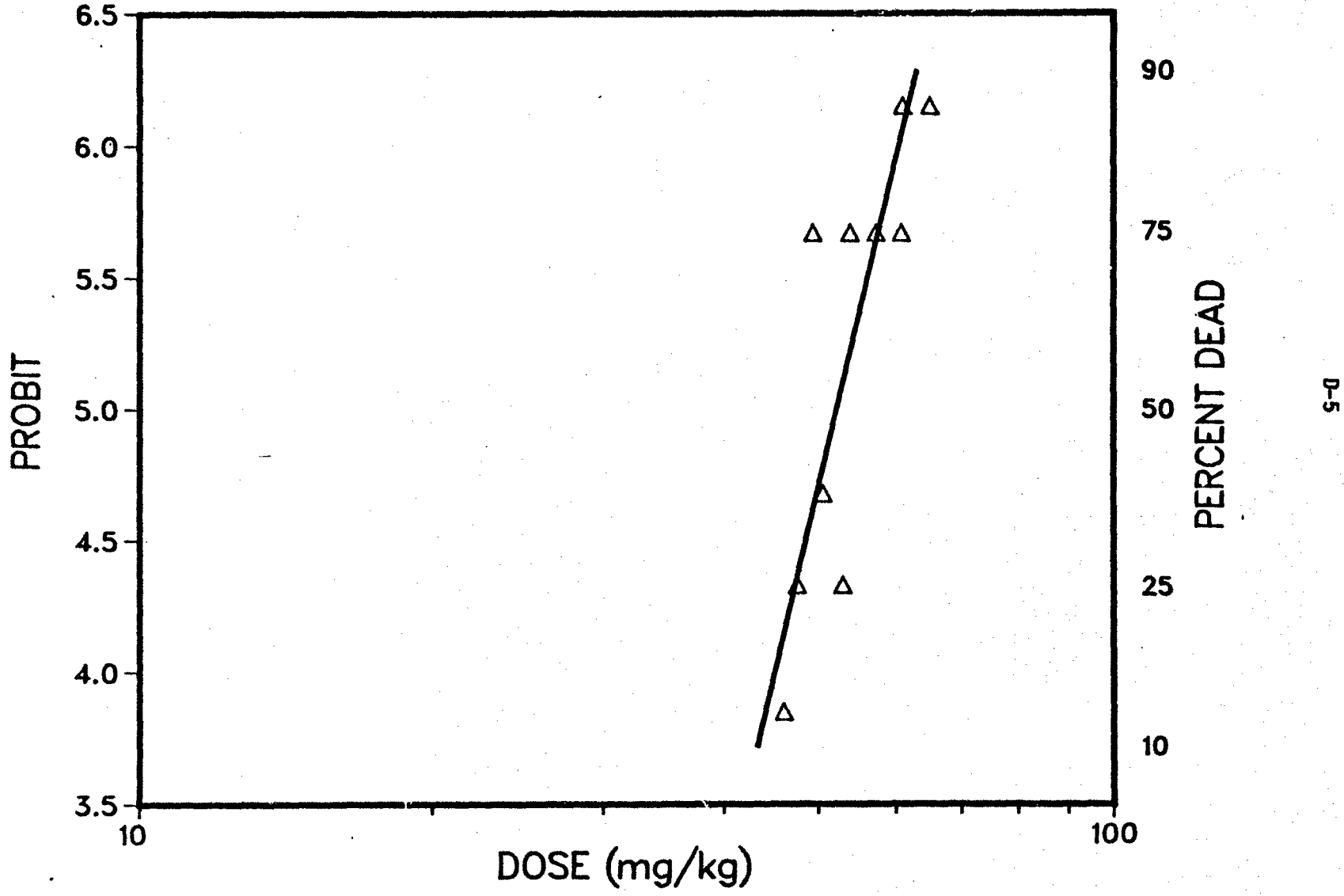


FIGURE 3.2.1 WHOLE BLOOD ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

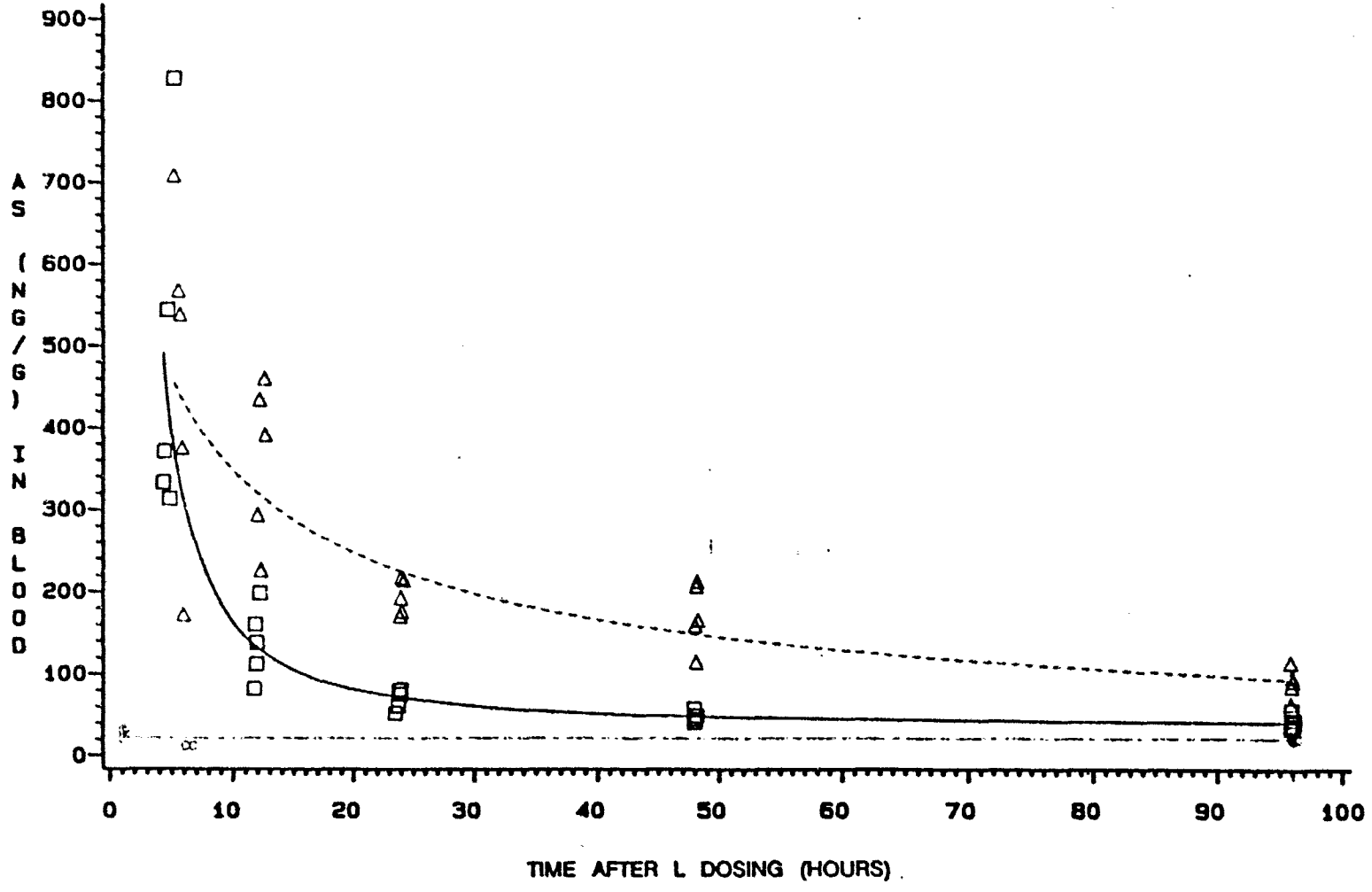


FIGURE 3.2.2 BRAIN ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

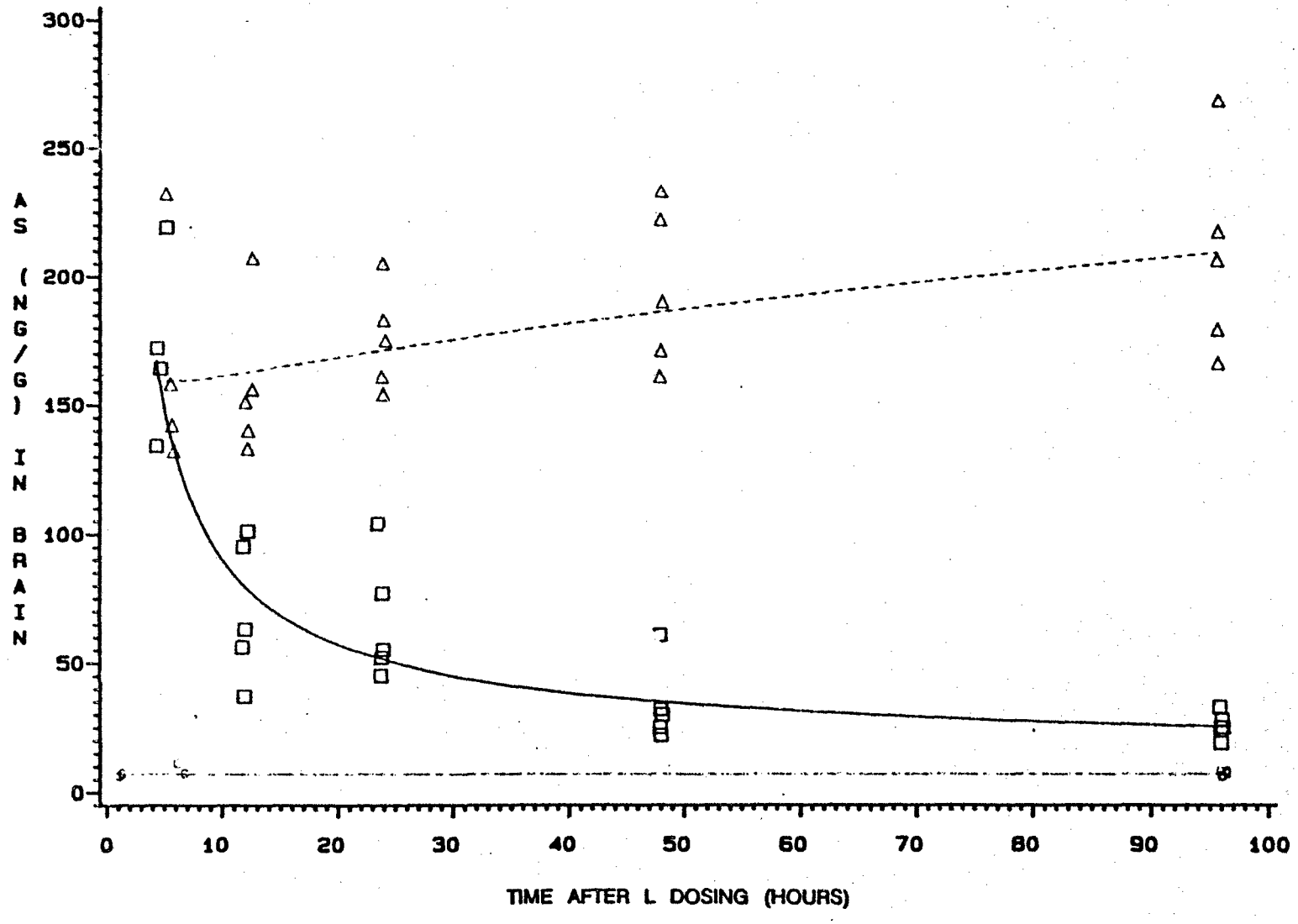


FIGURE 3.2.3 SPINAL CORD ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

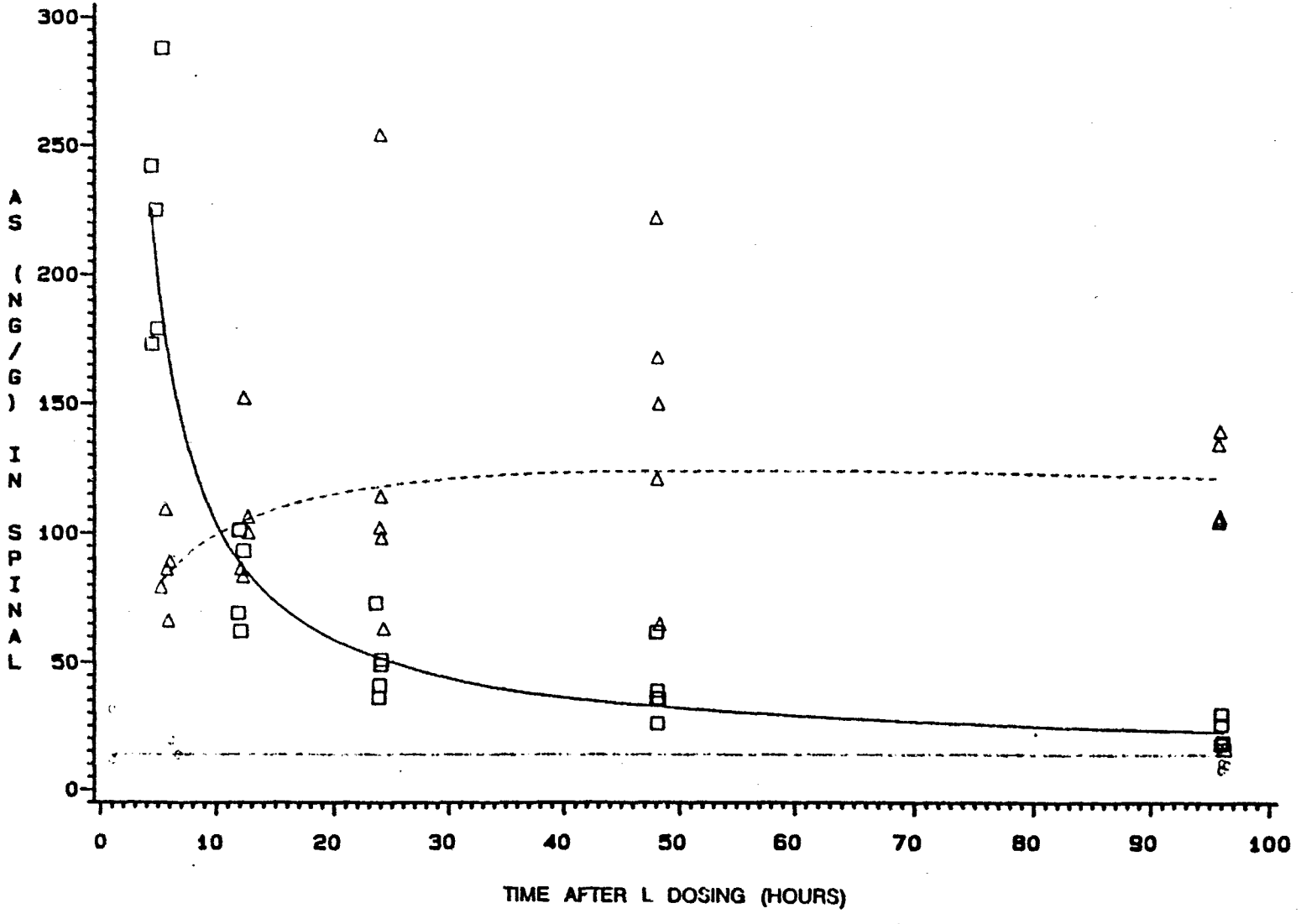
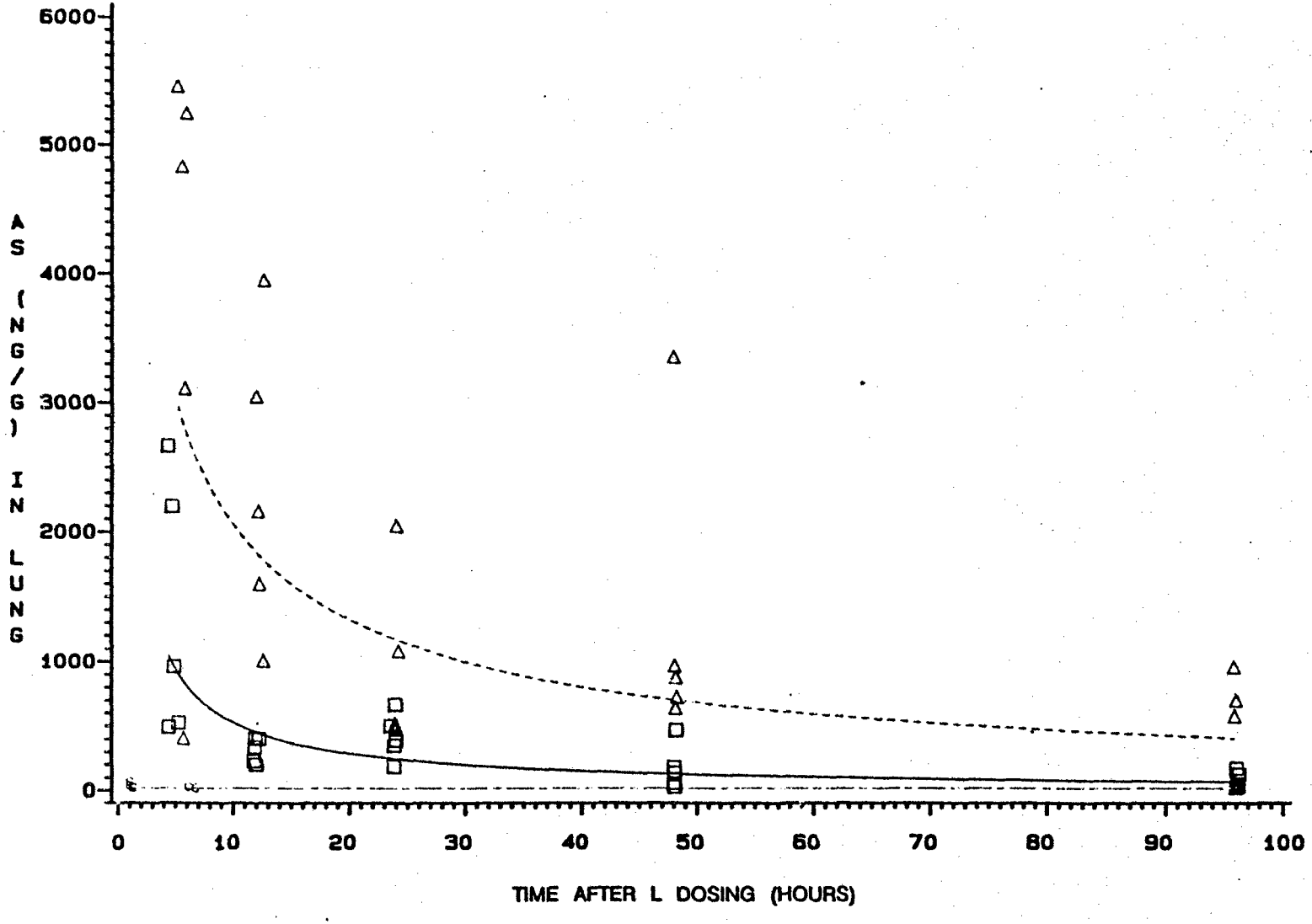
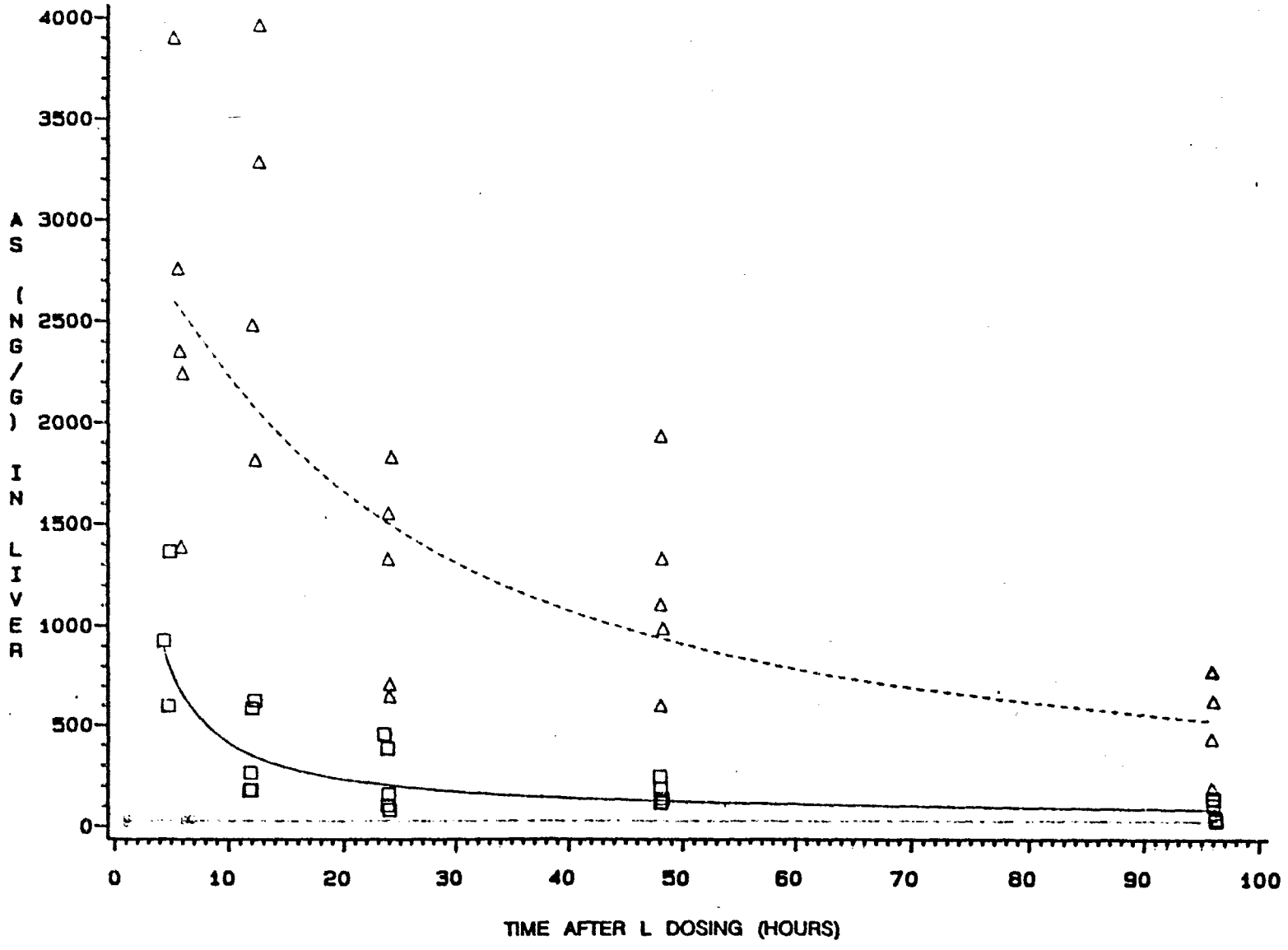


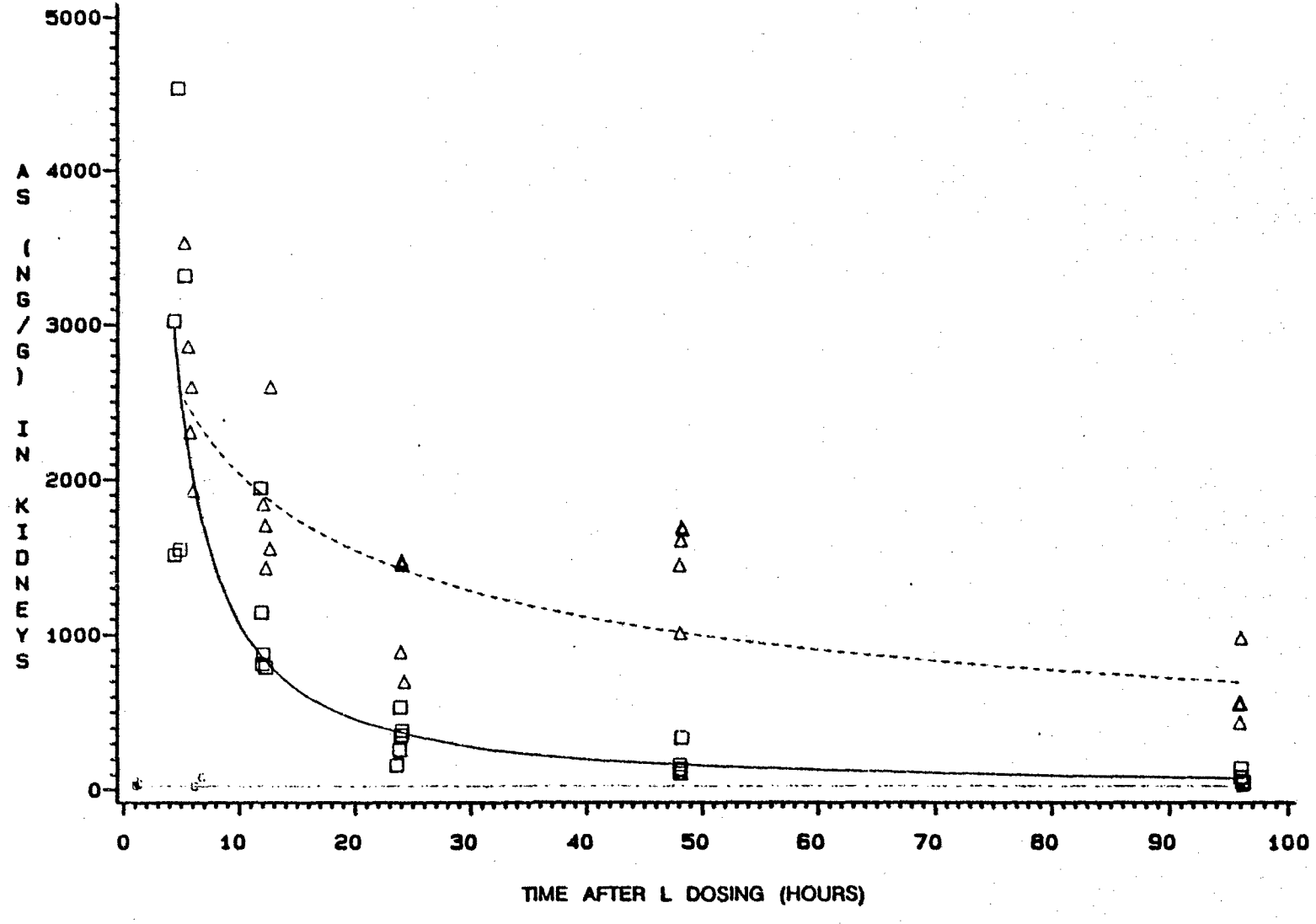
FIGURE 3.2.4 RIGHT LUNG ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



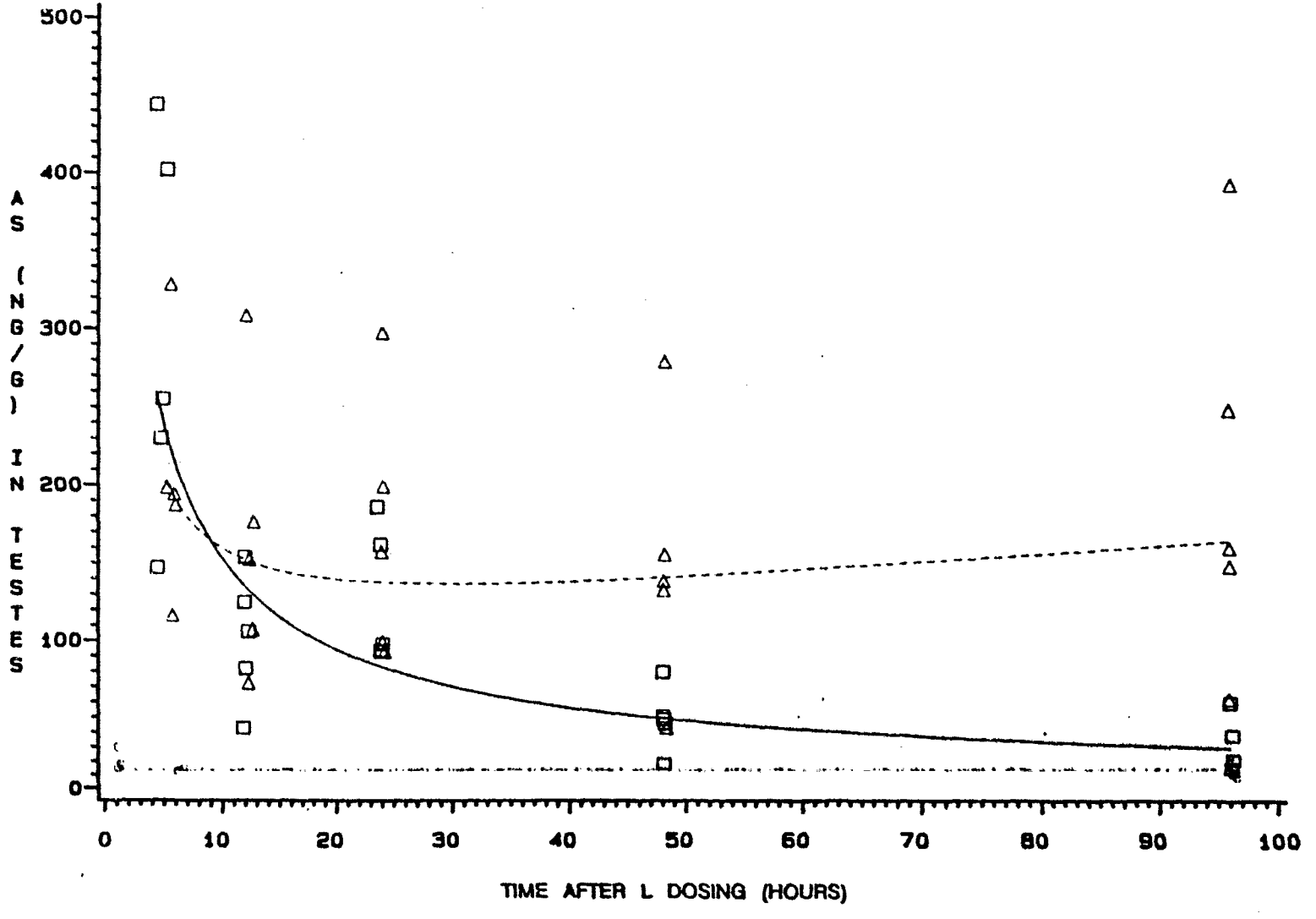
SUBCUTANEOUS ADMINISTRATION OF L AT THE LD10 (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



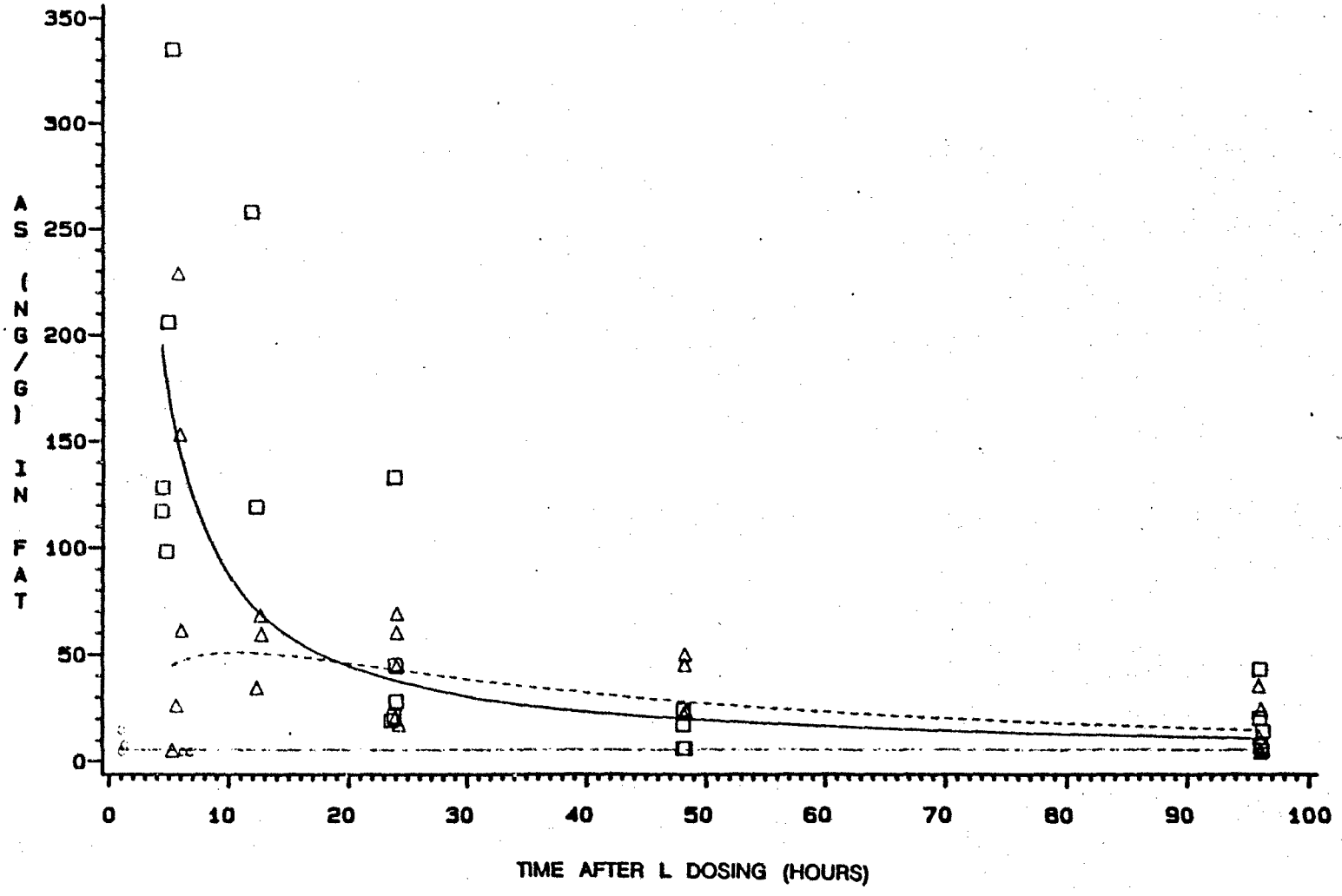
SUBCUTANEOUS ADMINISTRATION OF L AT THE LD10 (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg)
WITH AND WITHOUT BAL THERAPY IN RABBITS



FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg)
WITH AND WITHOUT BAL THERAPY IN RABBITS



P-13

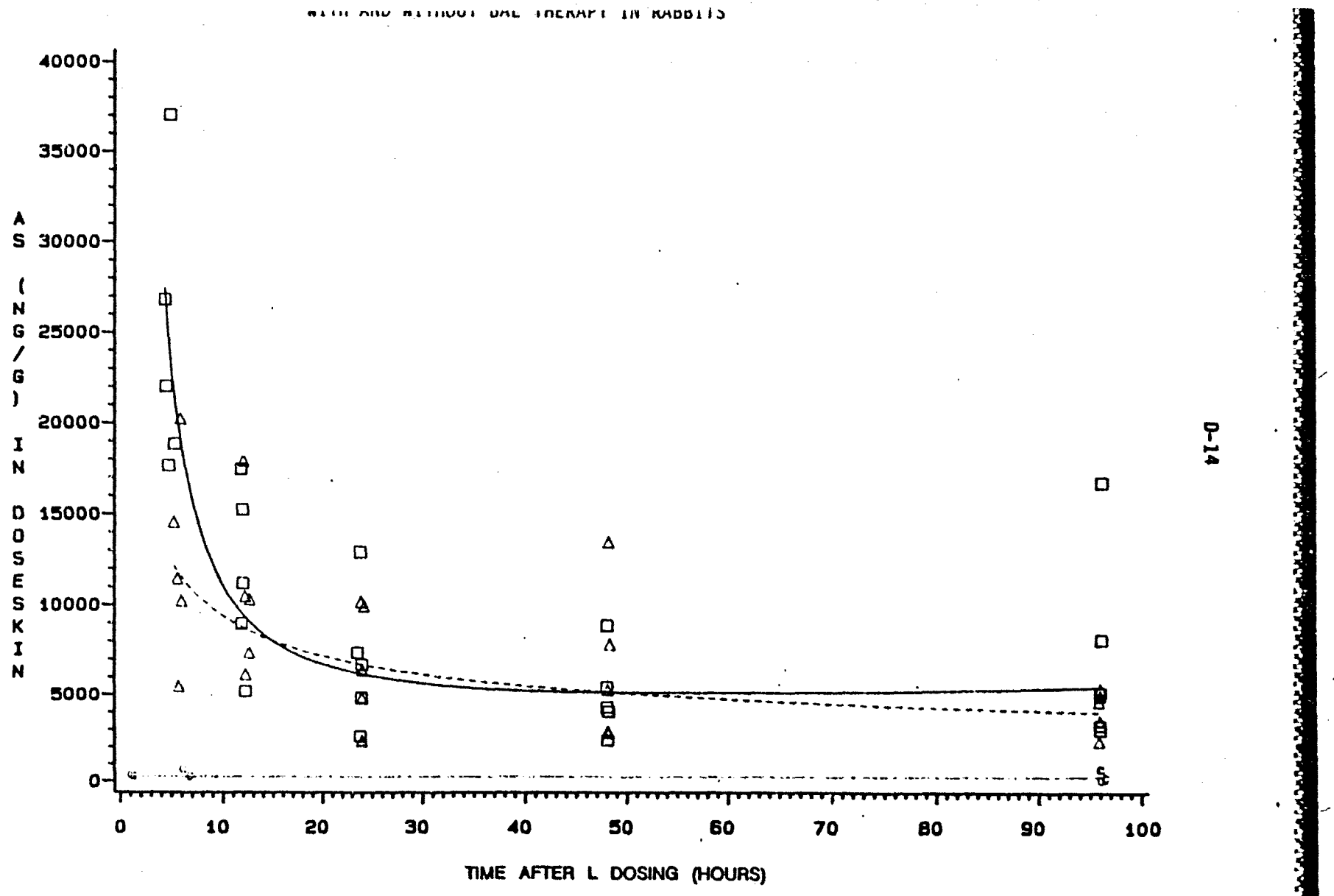

AS (NG/G) IN DOSE SKIN
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
70
80
90
100
TIME AFTER L DOSING (HOURS)

FIGURE 3.2.10 NORMAL SKIN ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD10 (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

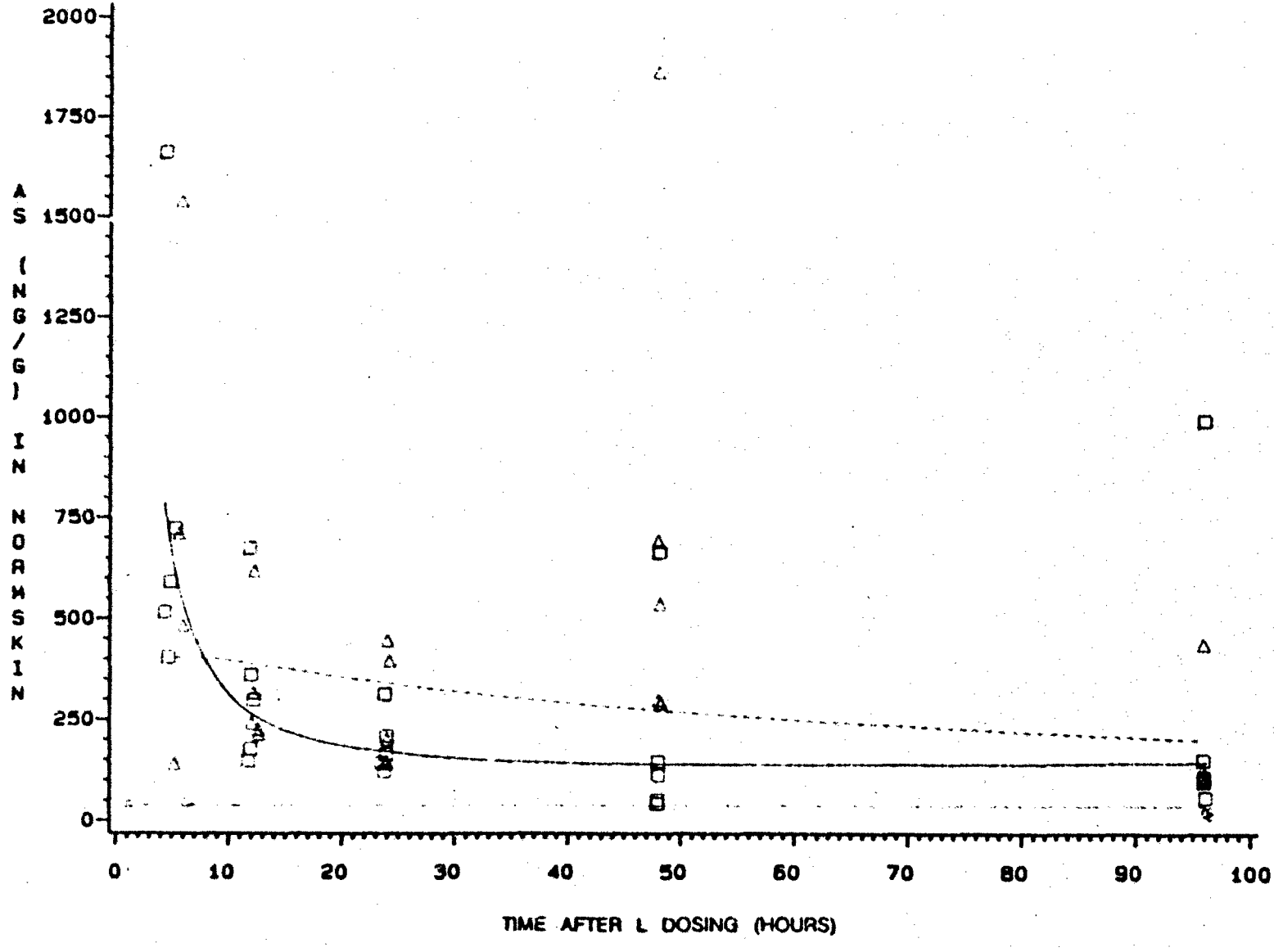


FIGURE 3.2.11 WHOLE BRAIN ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

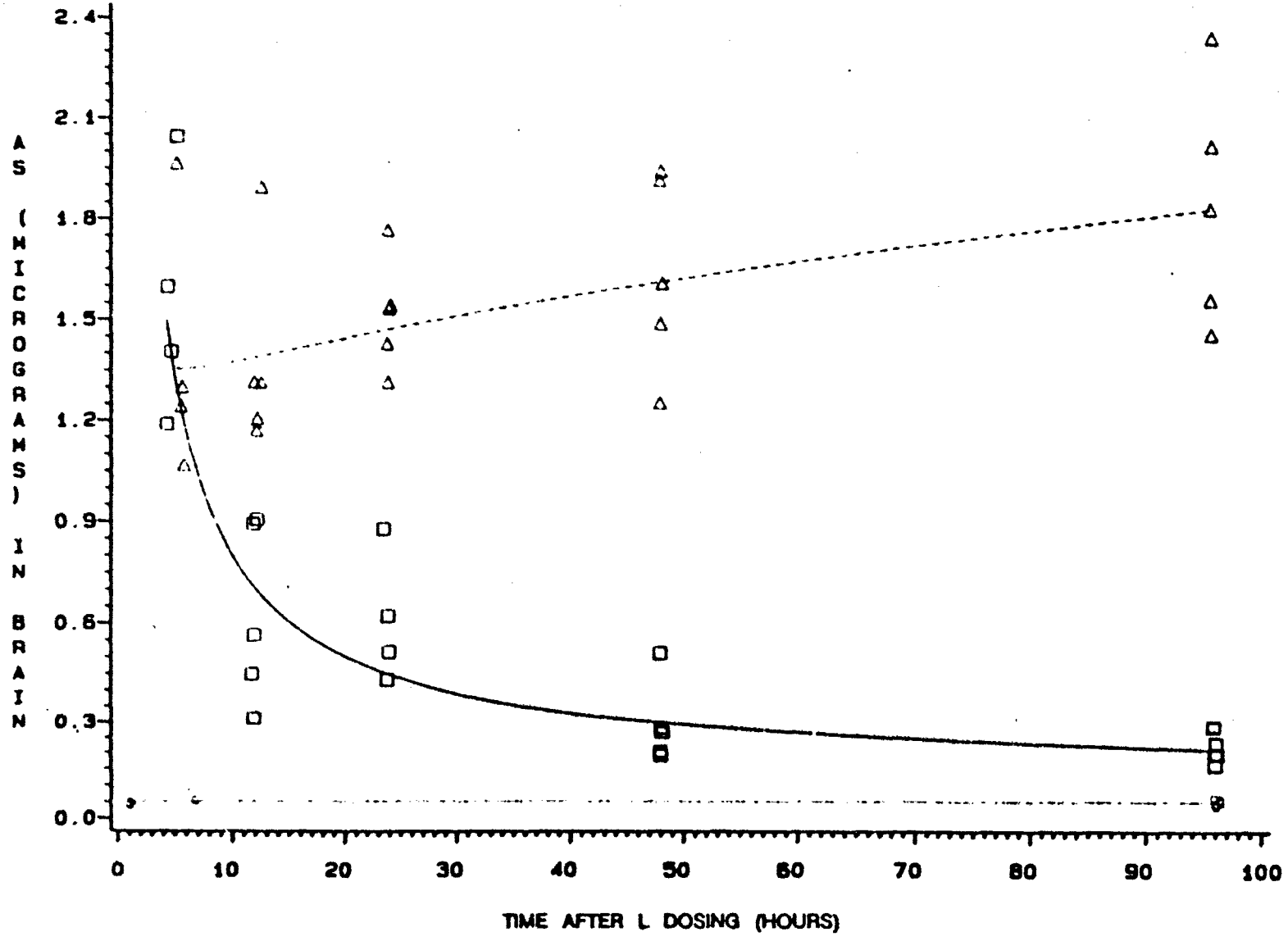


FIGURE 3.2.12 WHOLE LUNGS ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

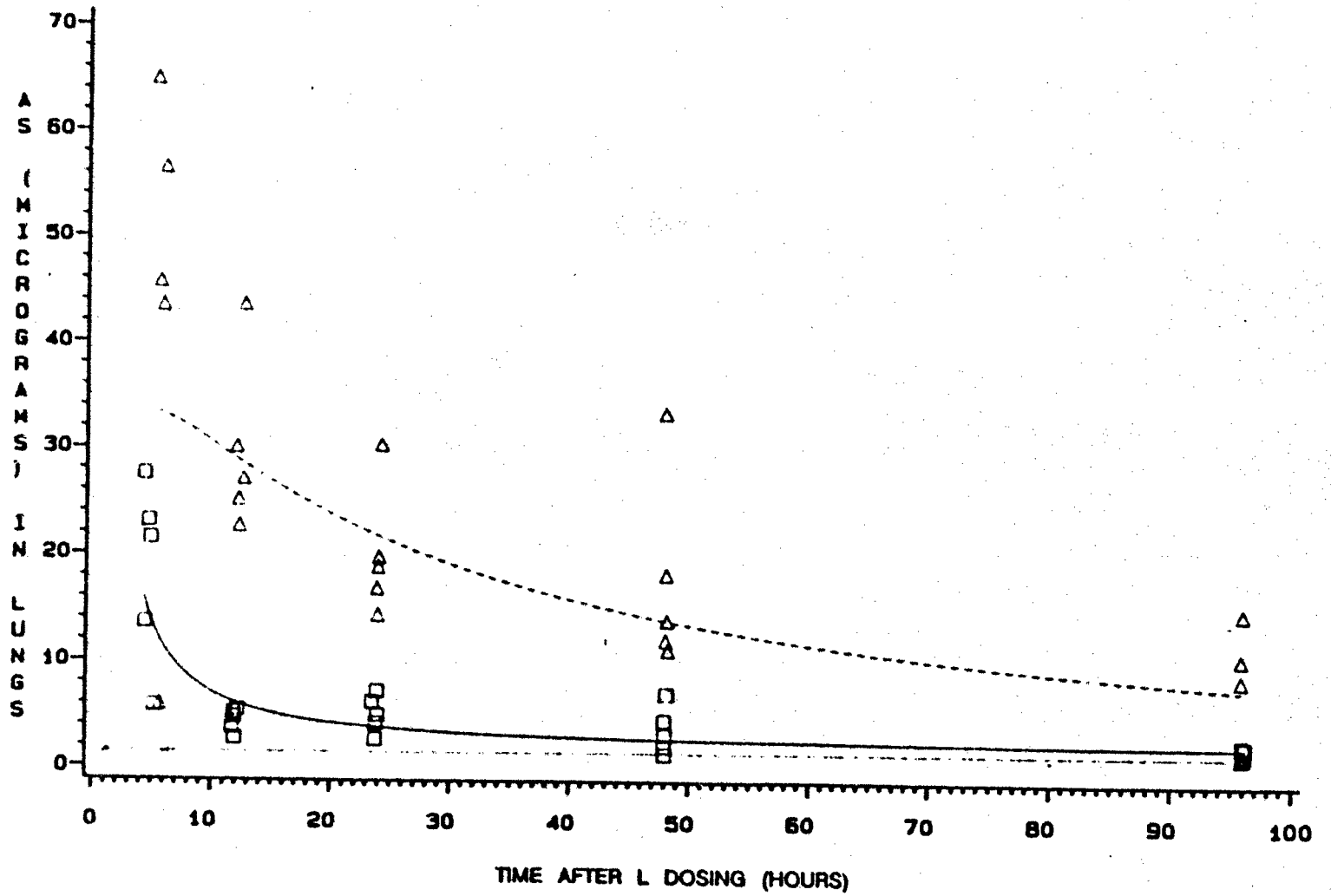


FIGURE 3.2.13 WHOLE LIVER ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

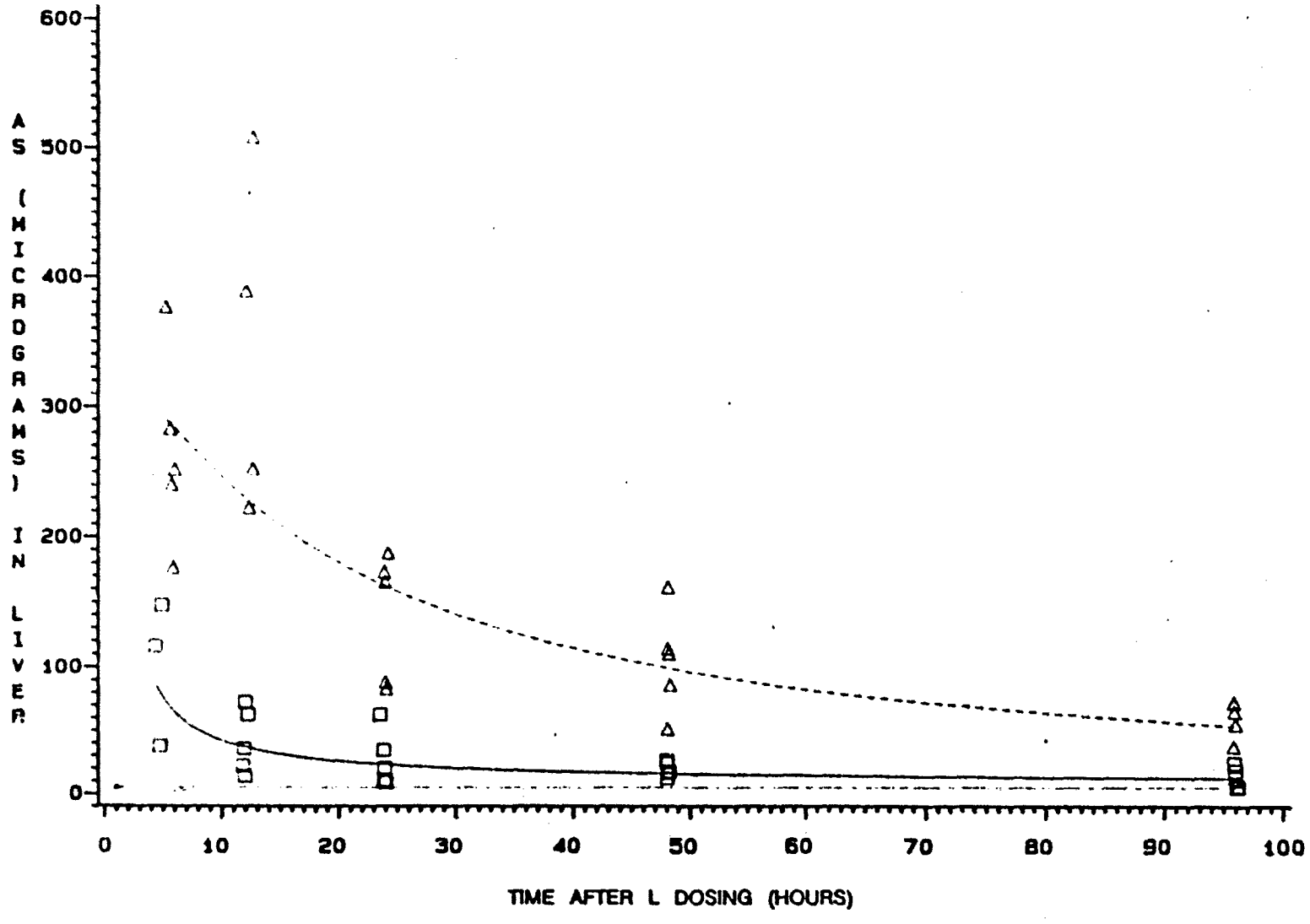


FIGURE 3.2.14 WHOLE KIDNEYS ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

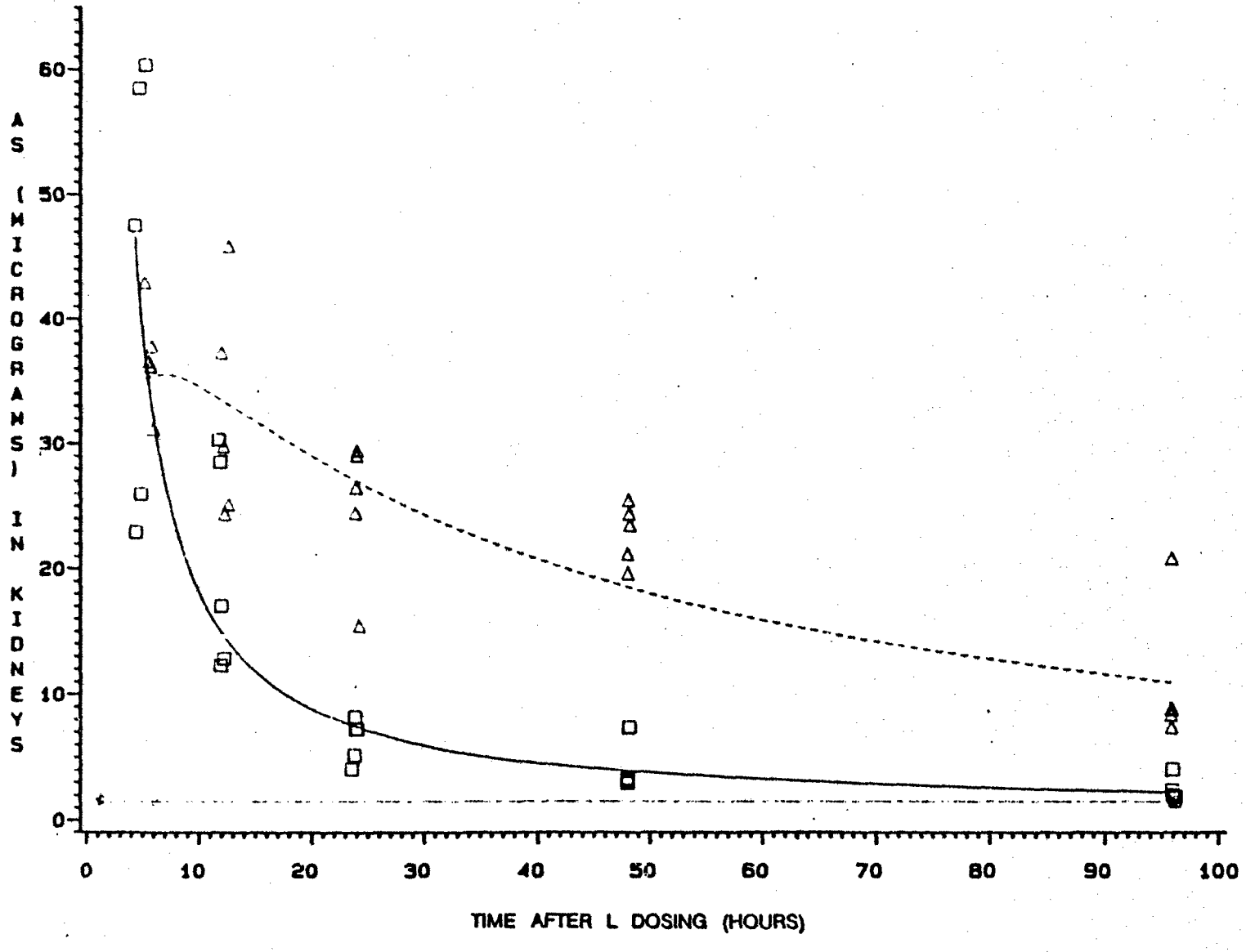


FIGURE 3.2.15 WHOLE TESTES ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

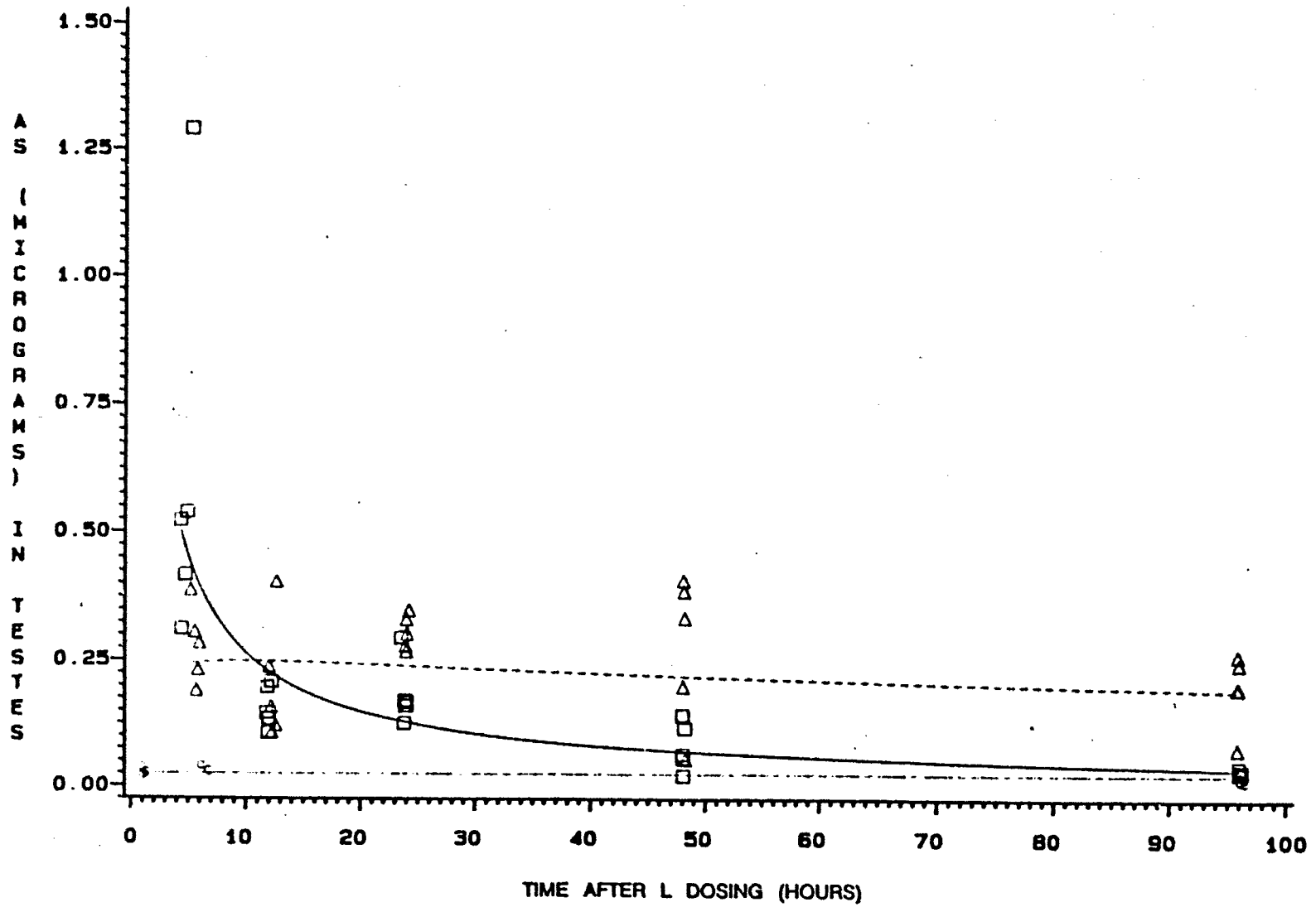


FIGURE 3.2.16 DOSE-SITE SKIN ARSENIC CONTENT (μg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{10} (2.4 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

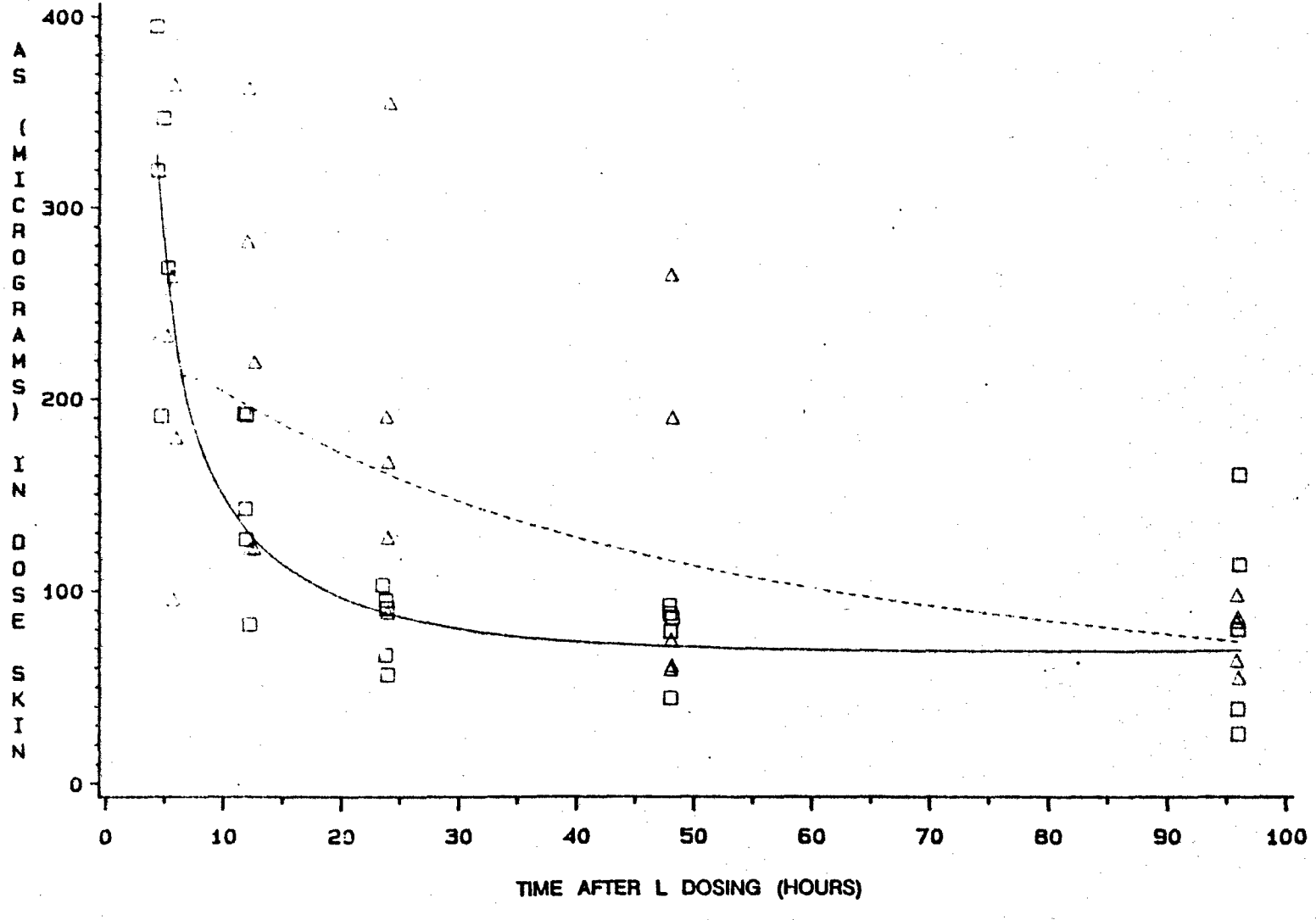
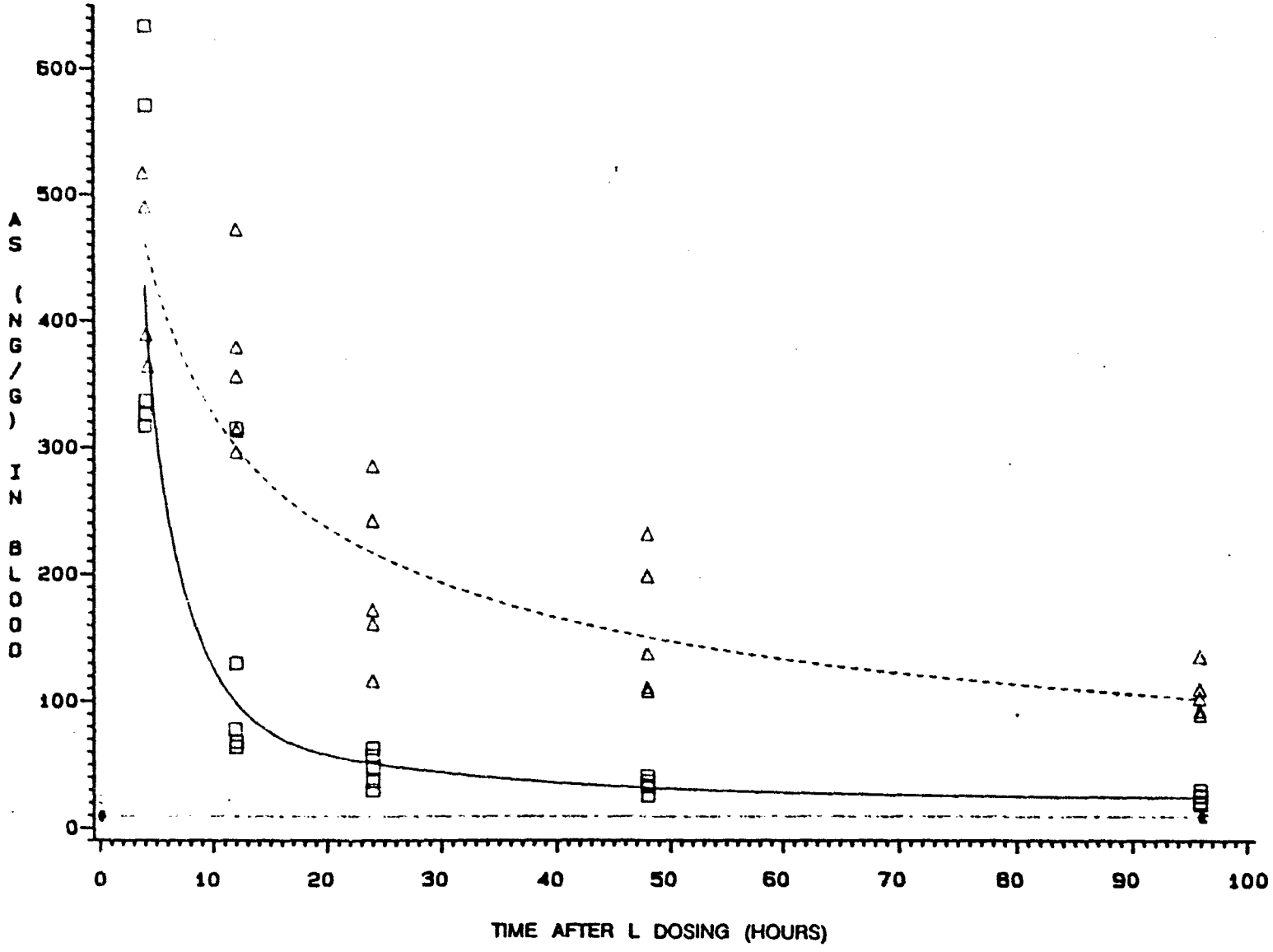
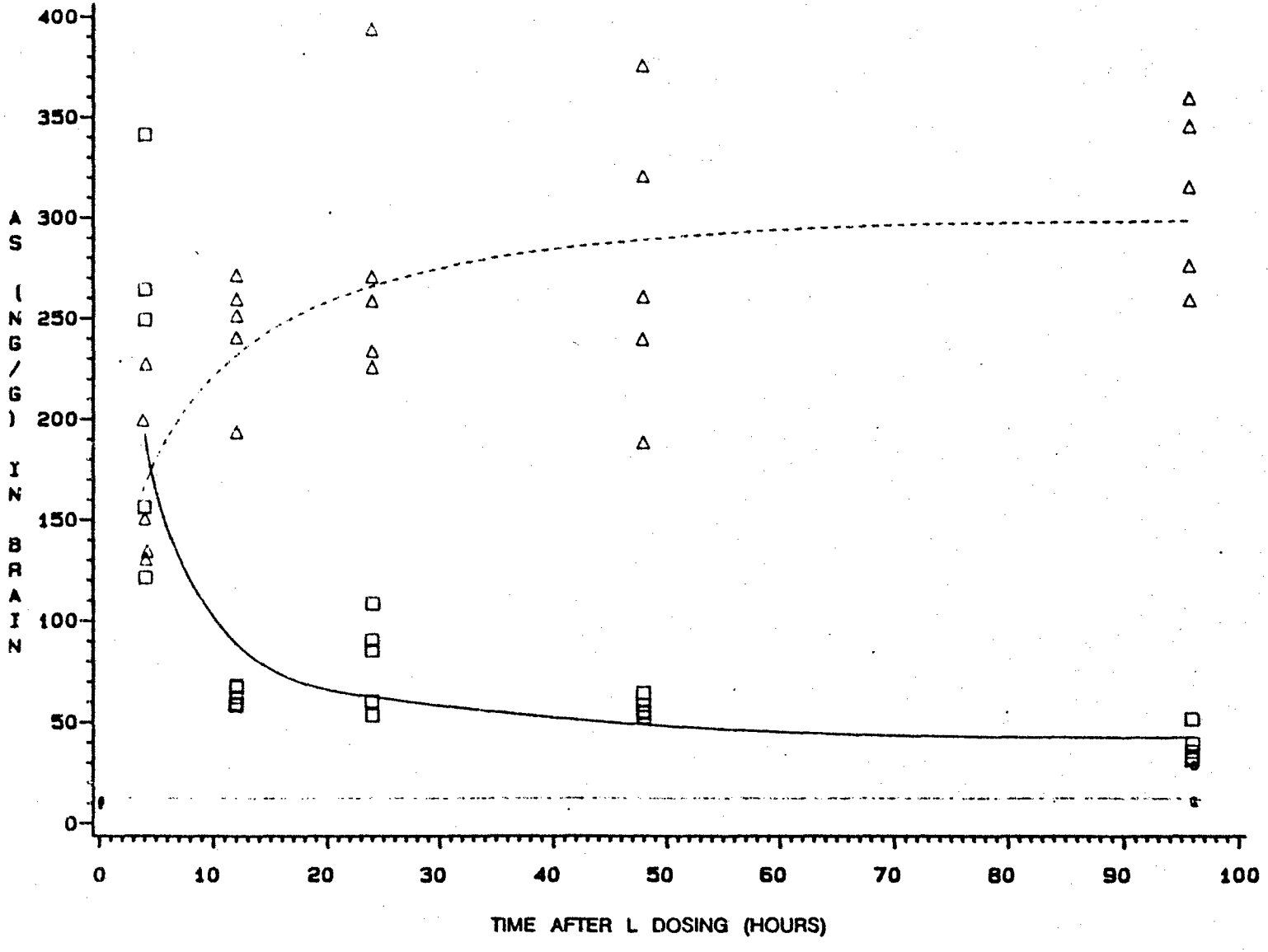


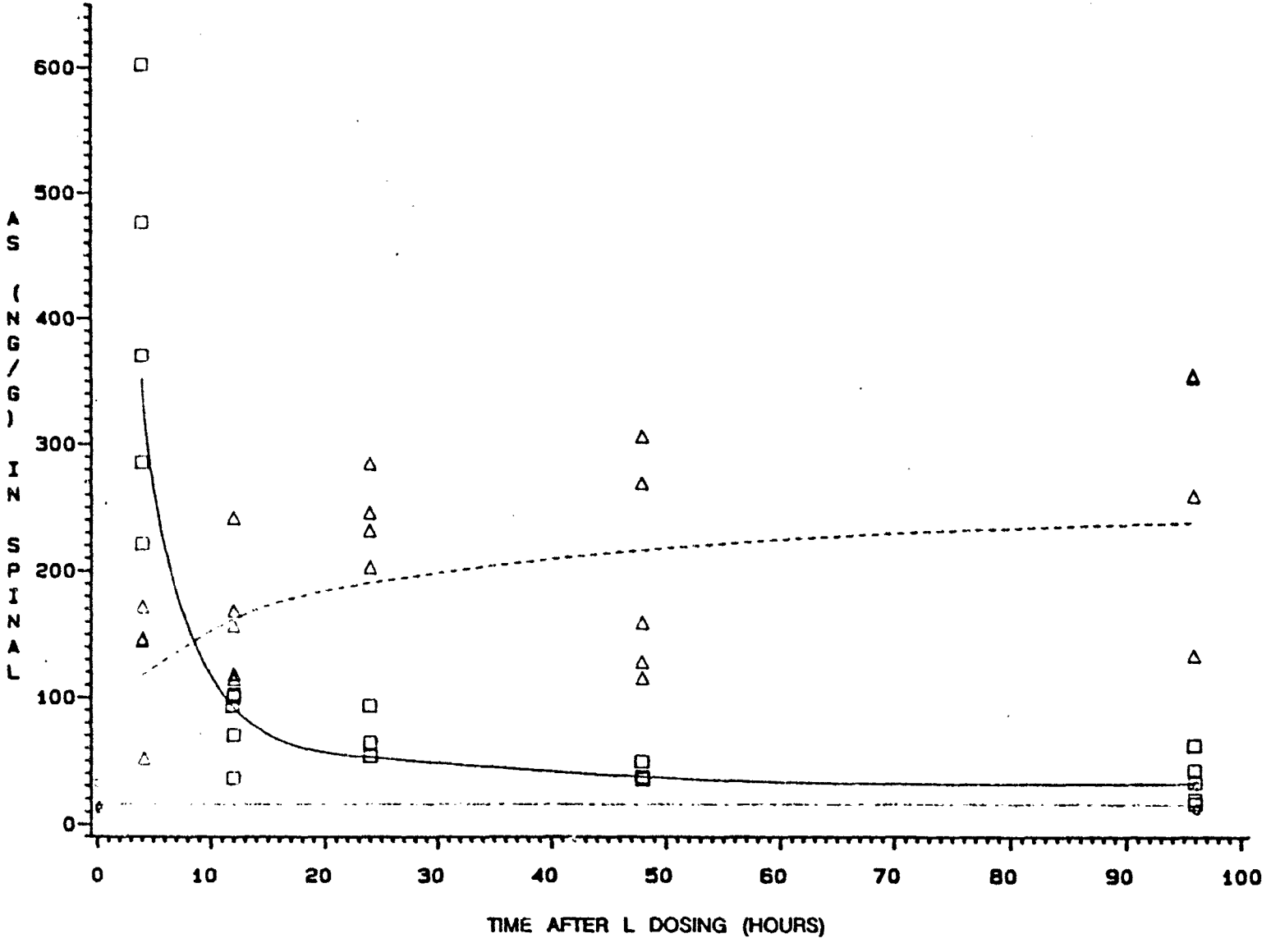
FIGURE 3.2.17 BLOOD ARSENIC CONCENTRATIONS (ng/g) AND ARSENIC REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



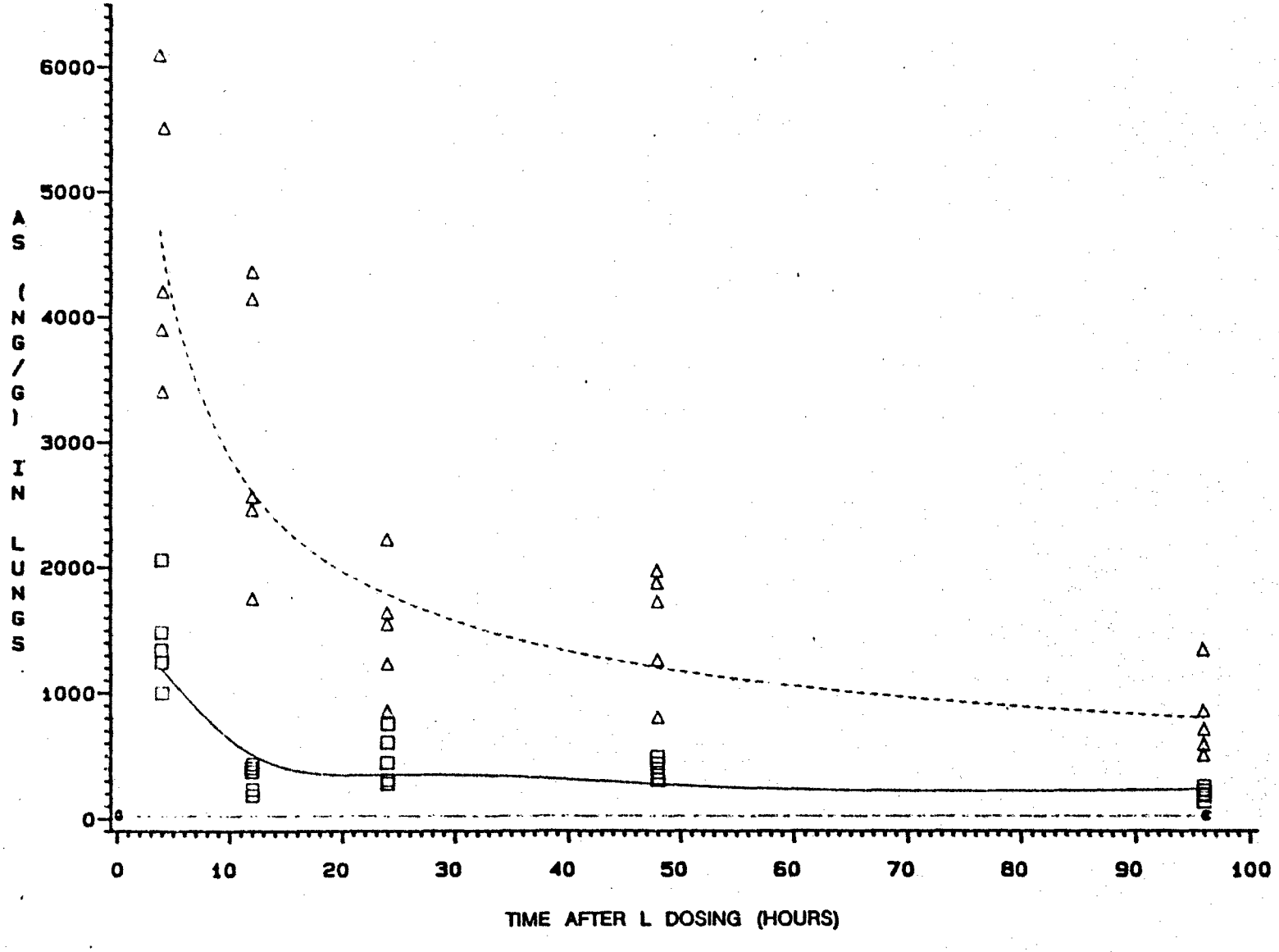
SUBCUTANEOUS ADMINISTRATION OF L AT THE LD40 (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



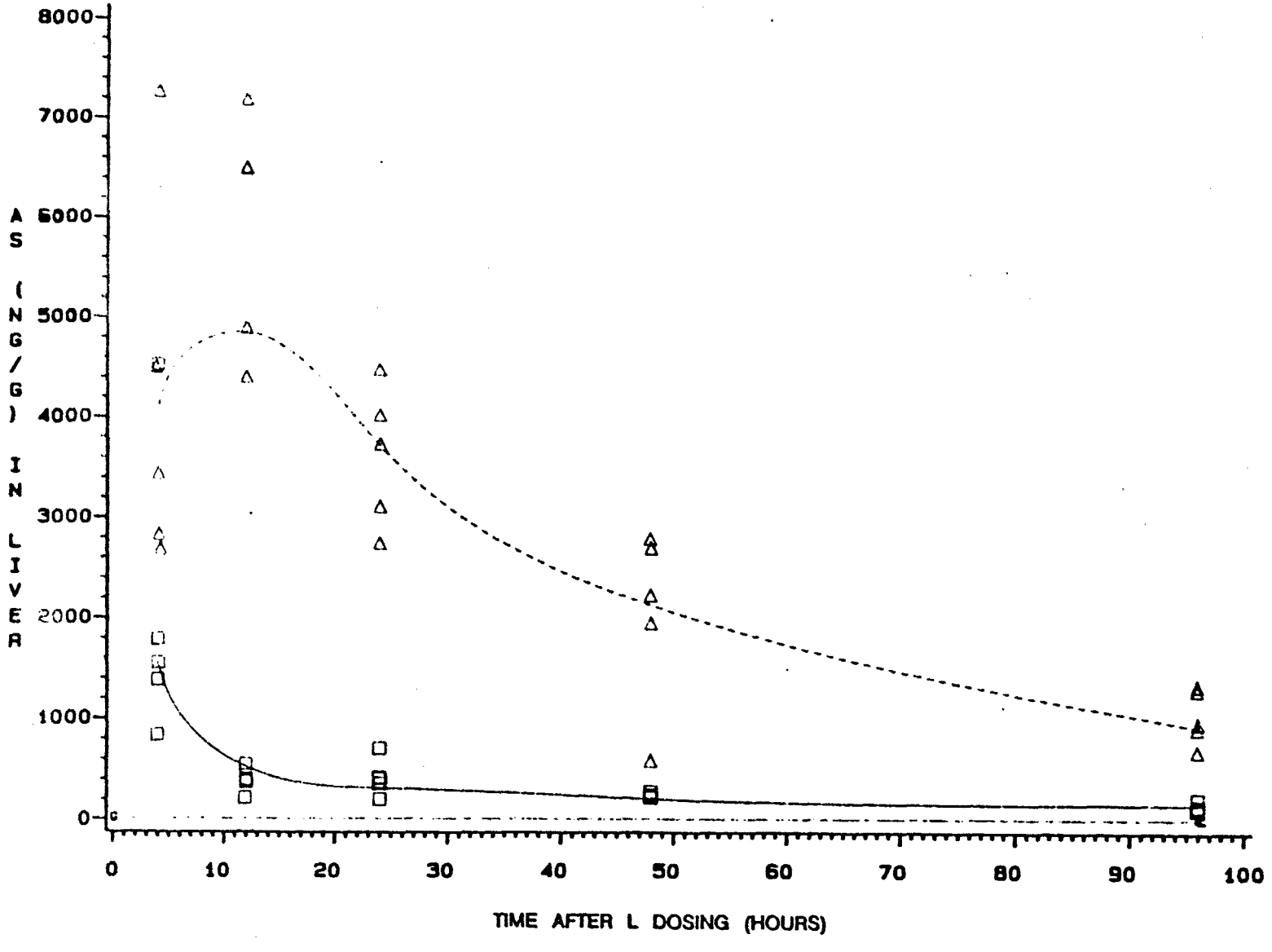
FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg)
WITH AND WITHOUT BAL THERAPY IN RABBITS



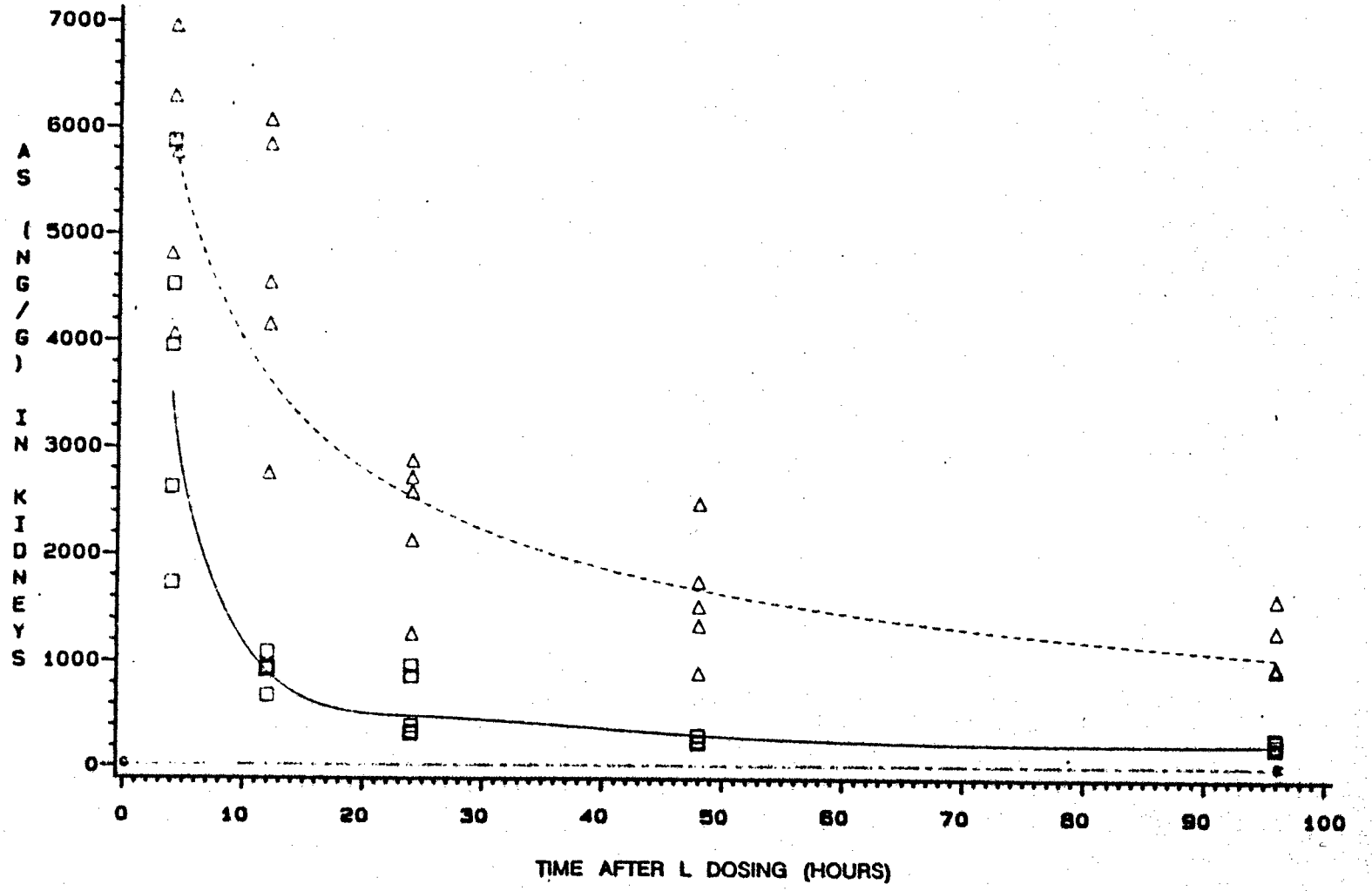
SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



WITHOUT BAL THERAPY IN RABBITS



WITHOUT BAL THERAPY IN RABBITS



WITH AND WITHOUT BAL THERAPY IN RABBITS

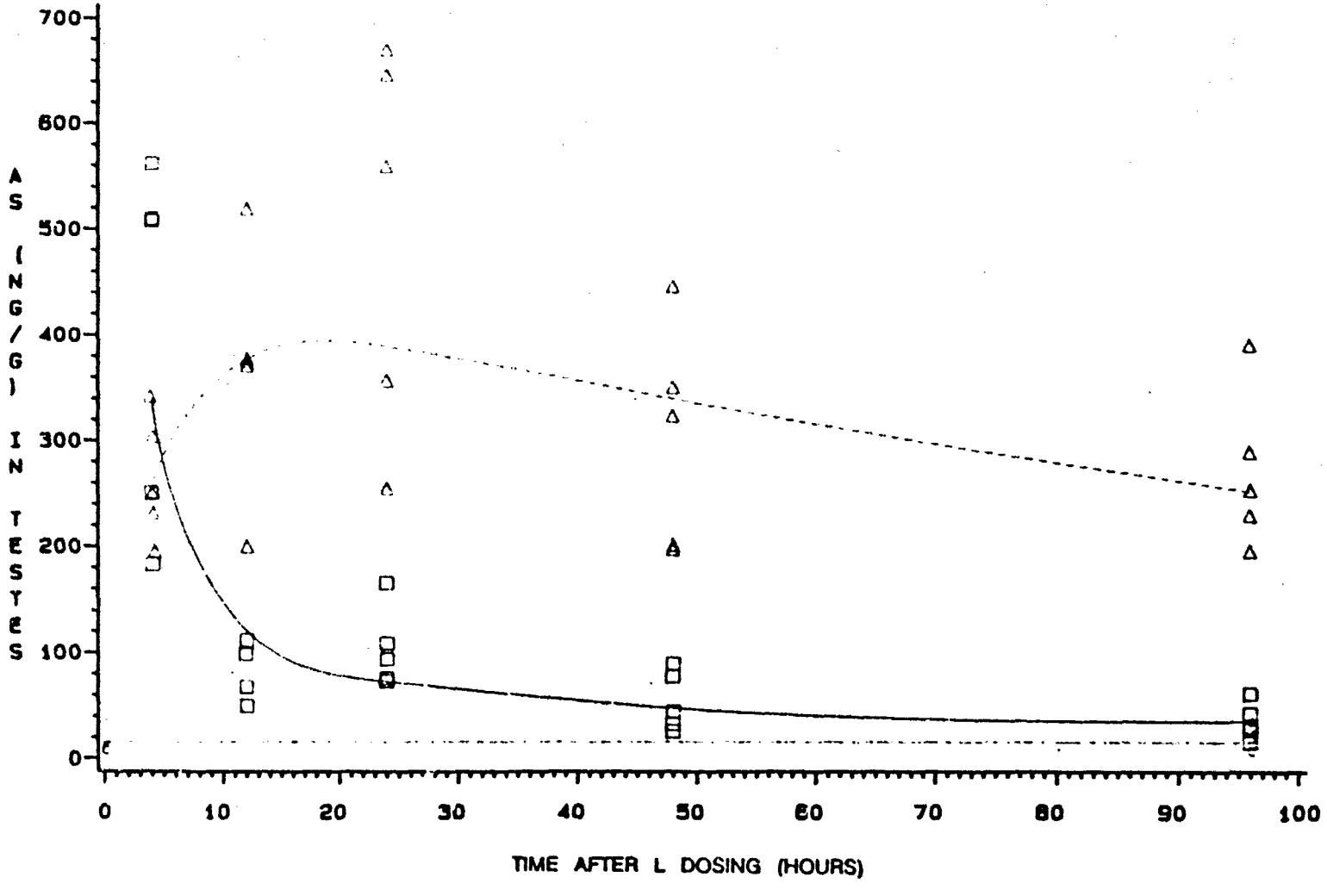


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FIGURE 3.2.24 ABDOMINAL FAT ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

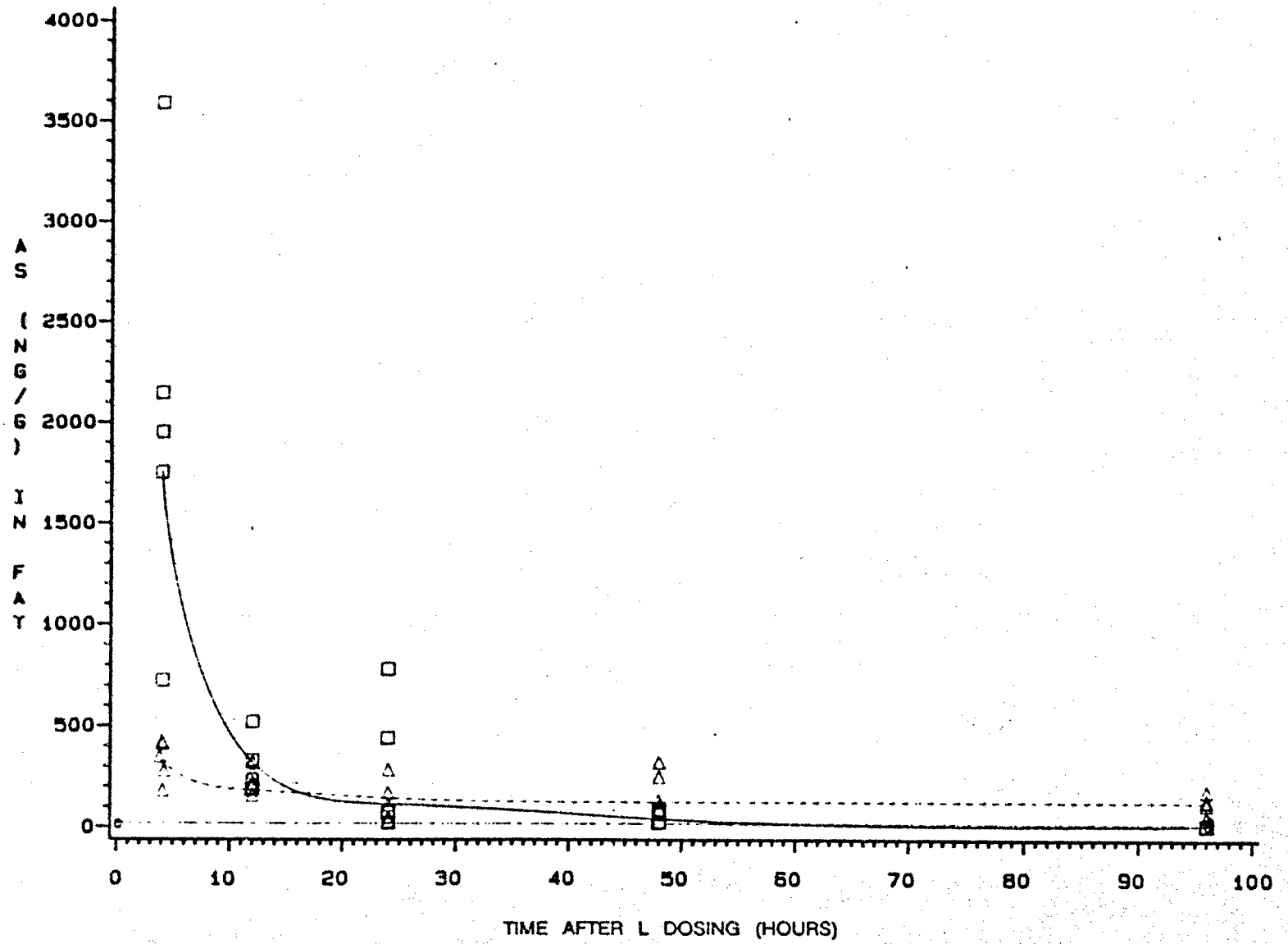


FIGURE 3.2.25 DOSE-SITE SKIN ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

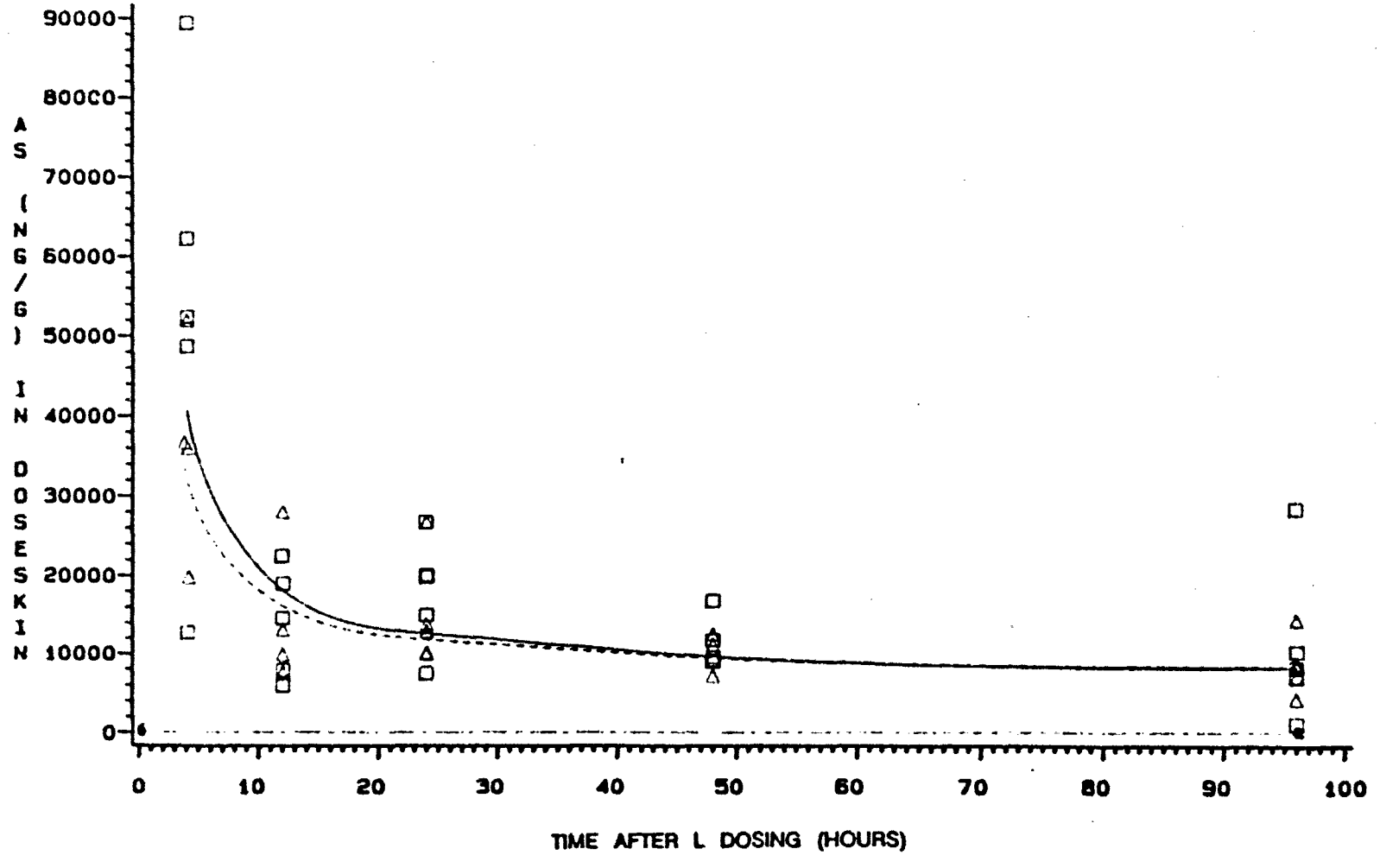


FIGURE 3.2.26 NORMAL SKIN ARSENIC CONCENTRATIONS (ng/g) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

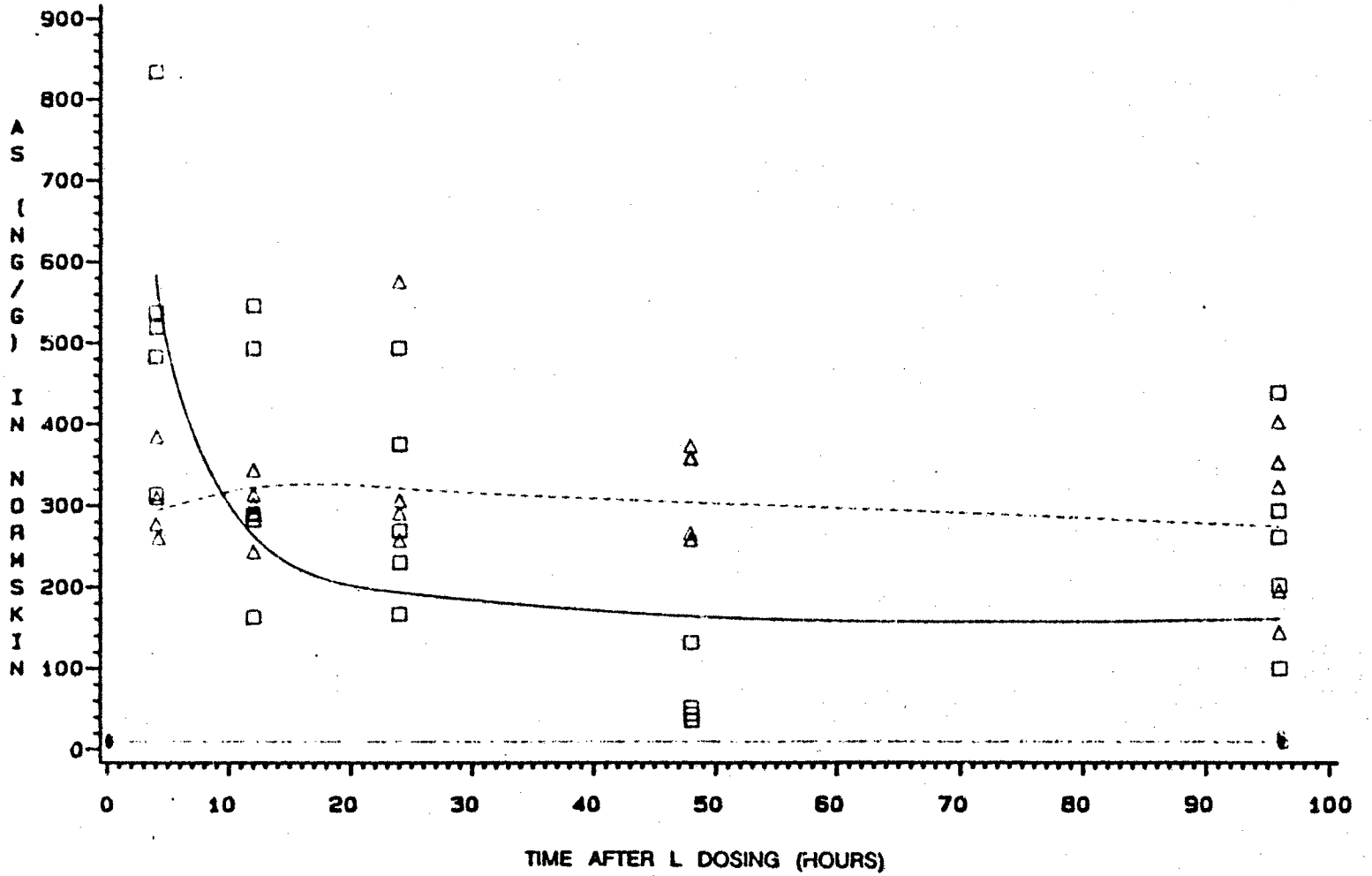


FIGURE 3.2.27 WHOLE BRAIN ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

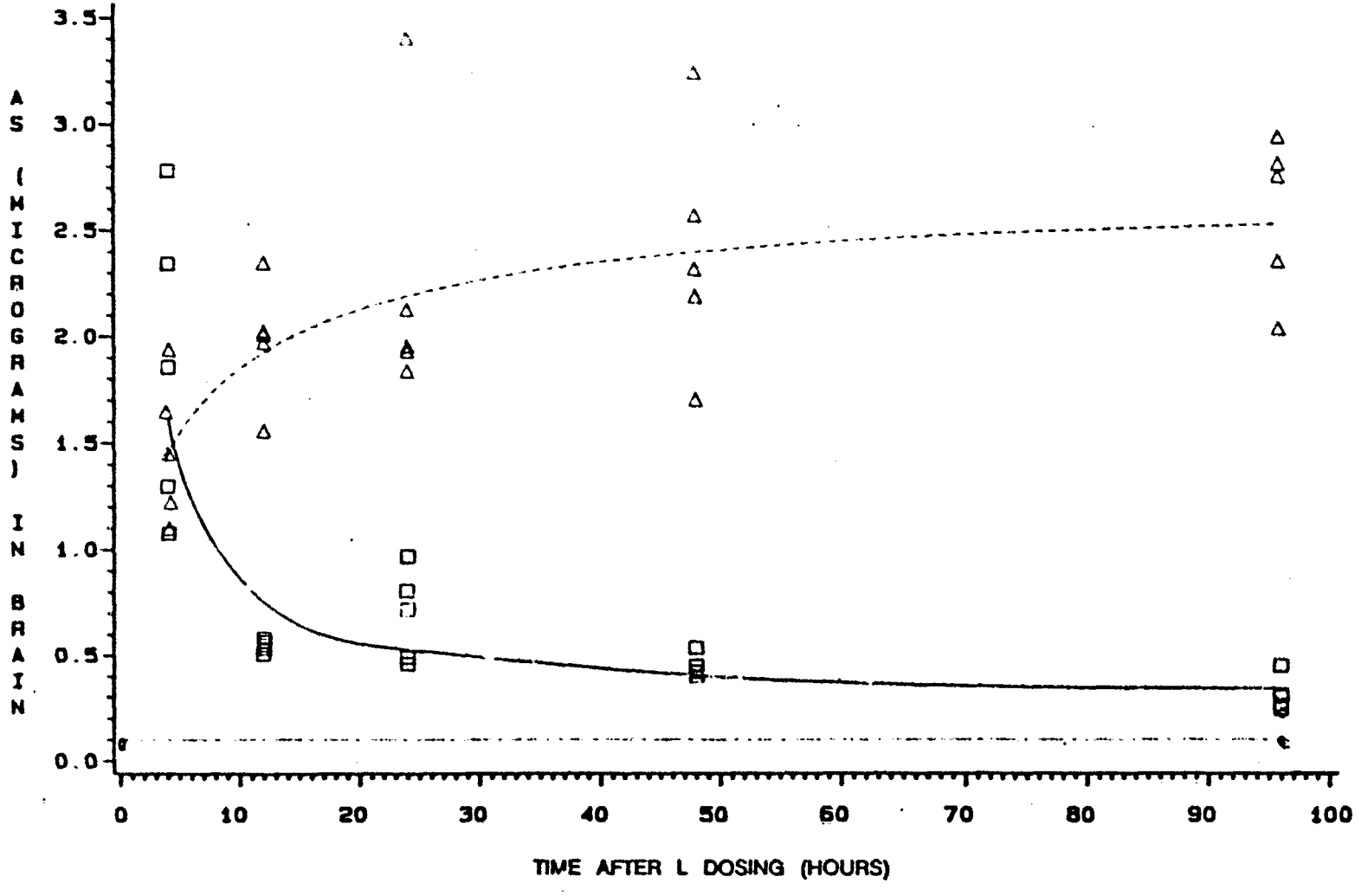


FIGURE 3.2.28 WHOLE LUNGS ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD 40 (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

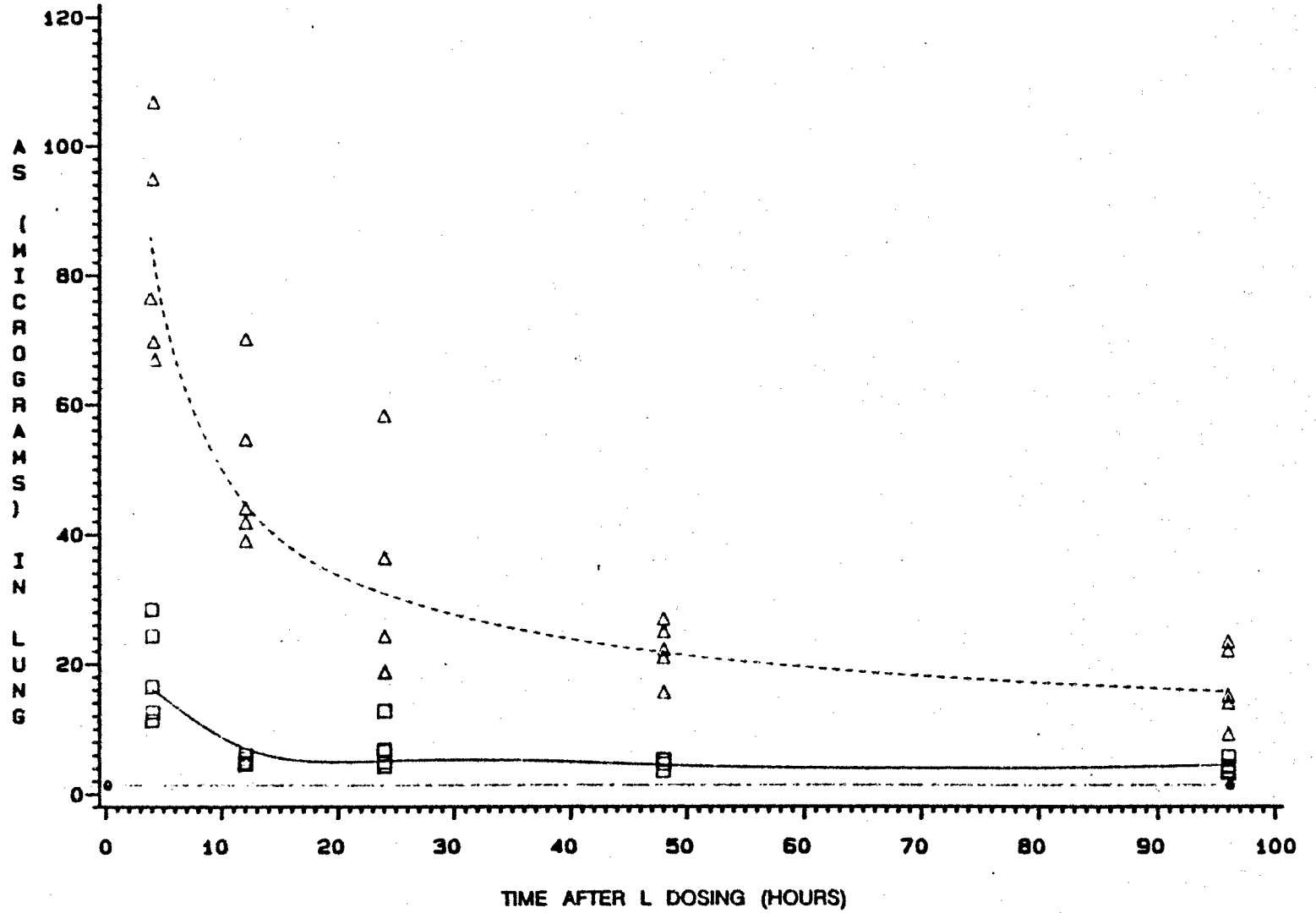


FIGURE 3.2.29 WHOLE LIVER ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD40 (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

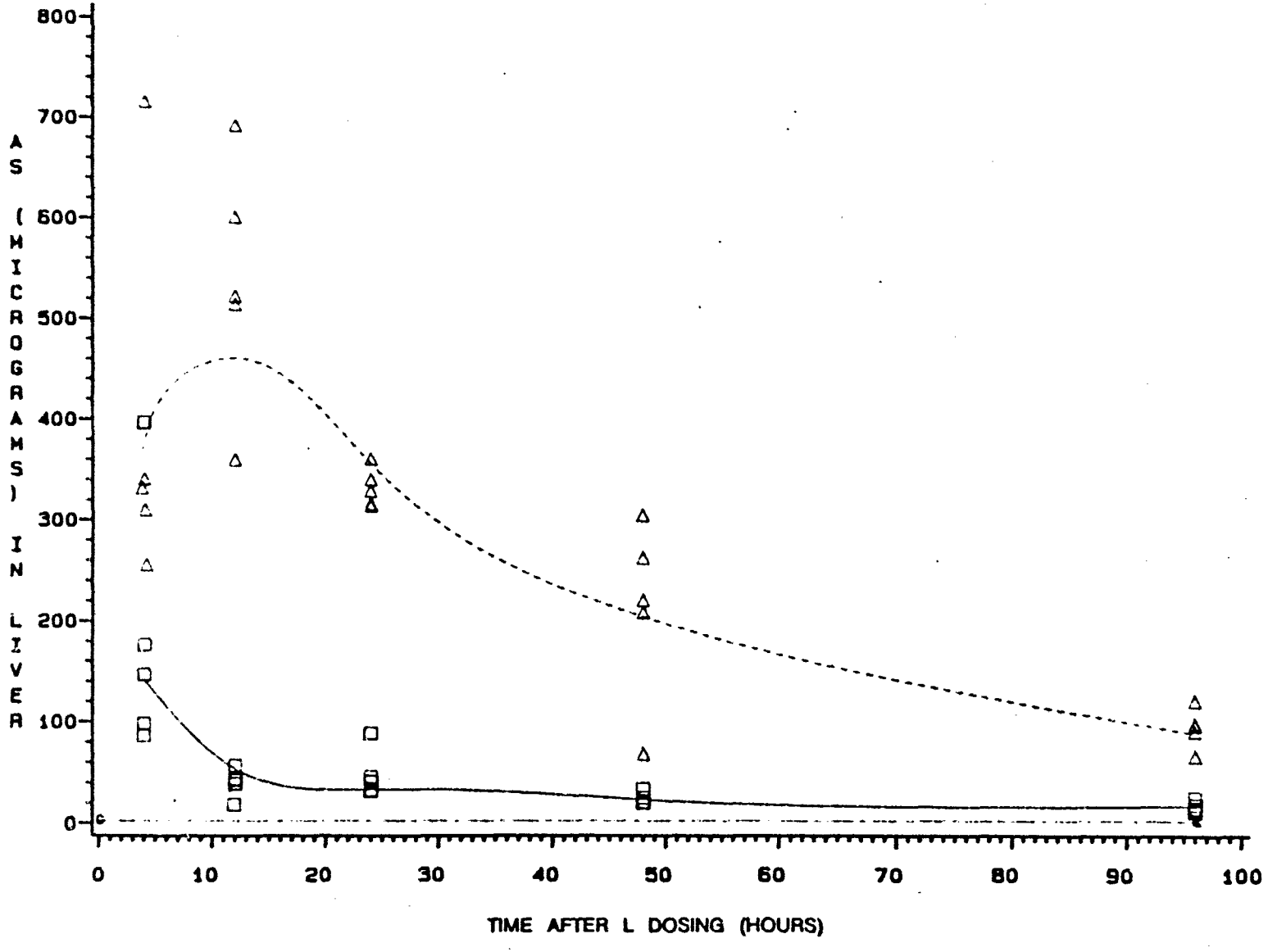


FIGURE 3.2.30 WHOLE KIDNEYS ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD40 (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

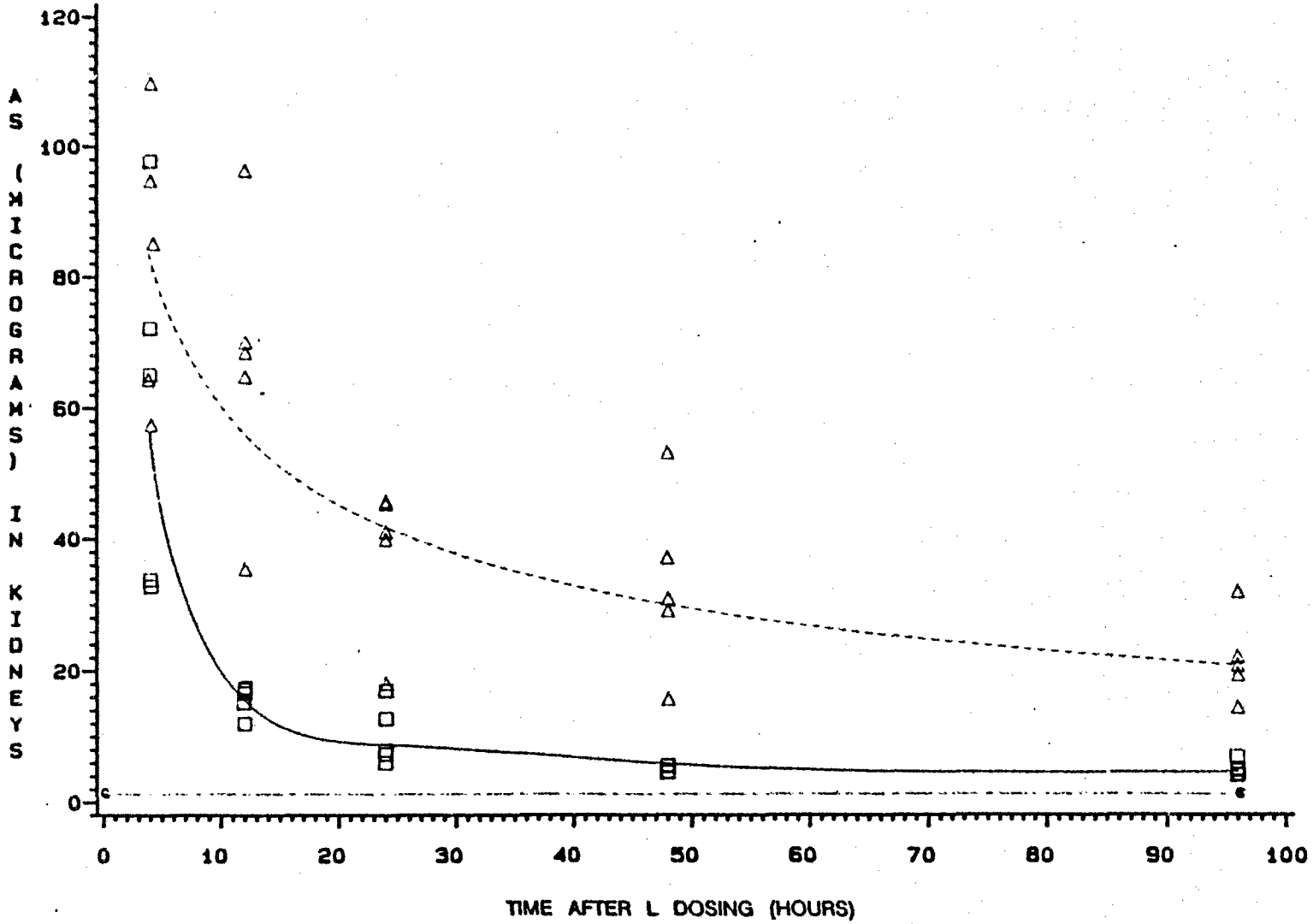
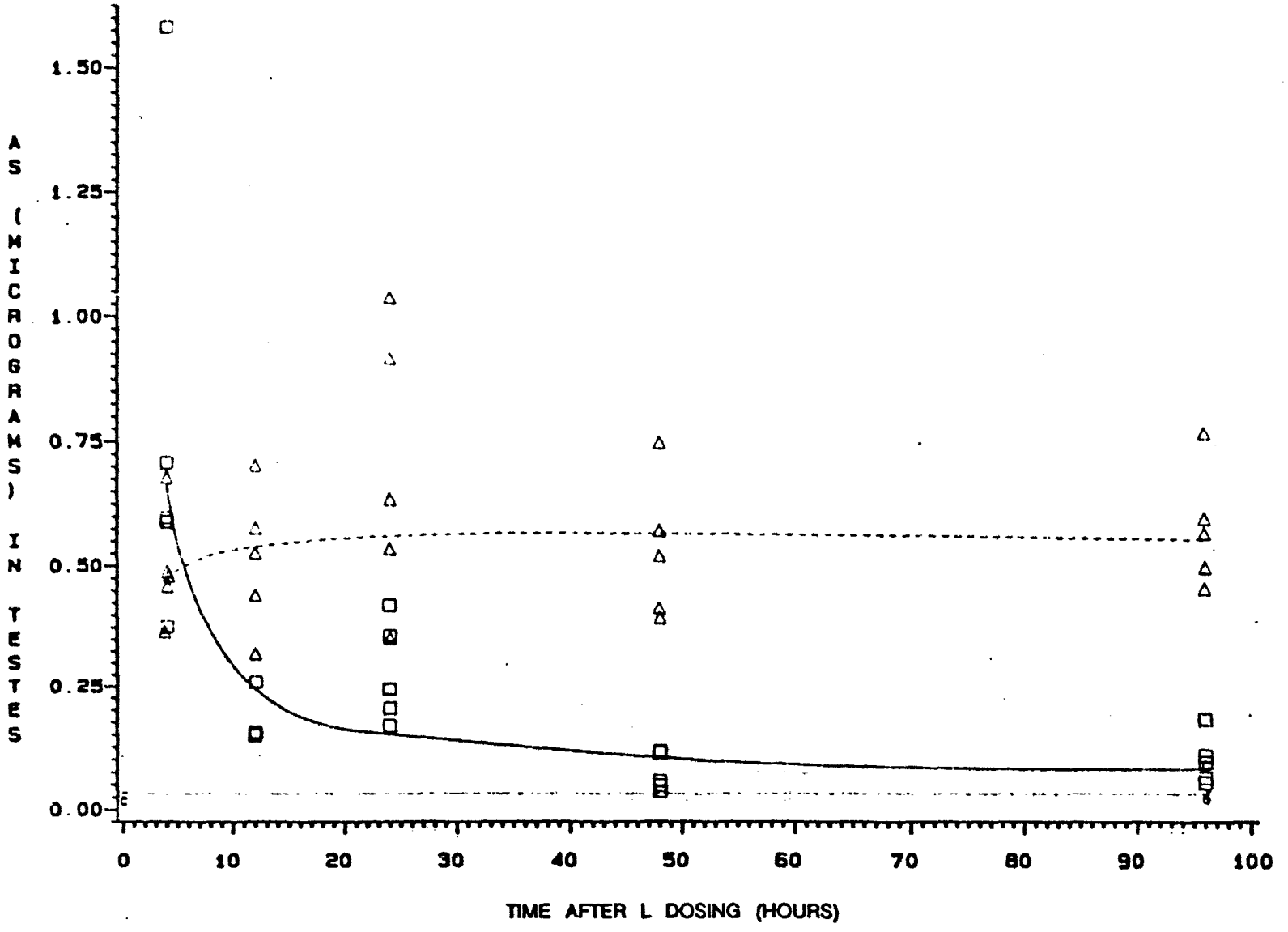


FIGURE 3.2.11 WHOLE TESTES ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



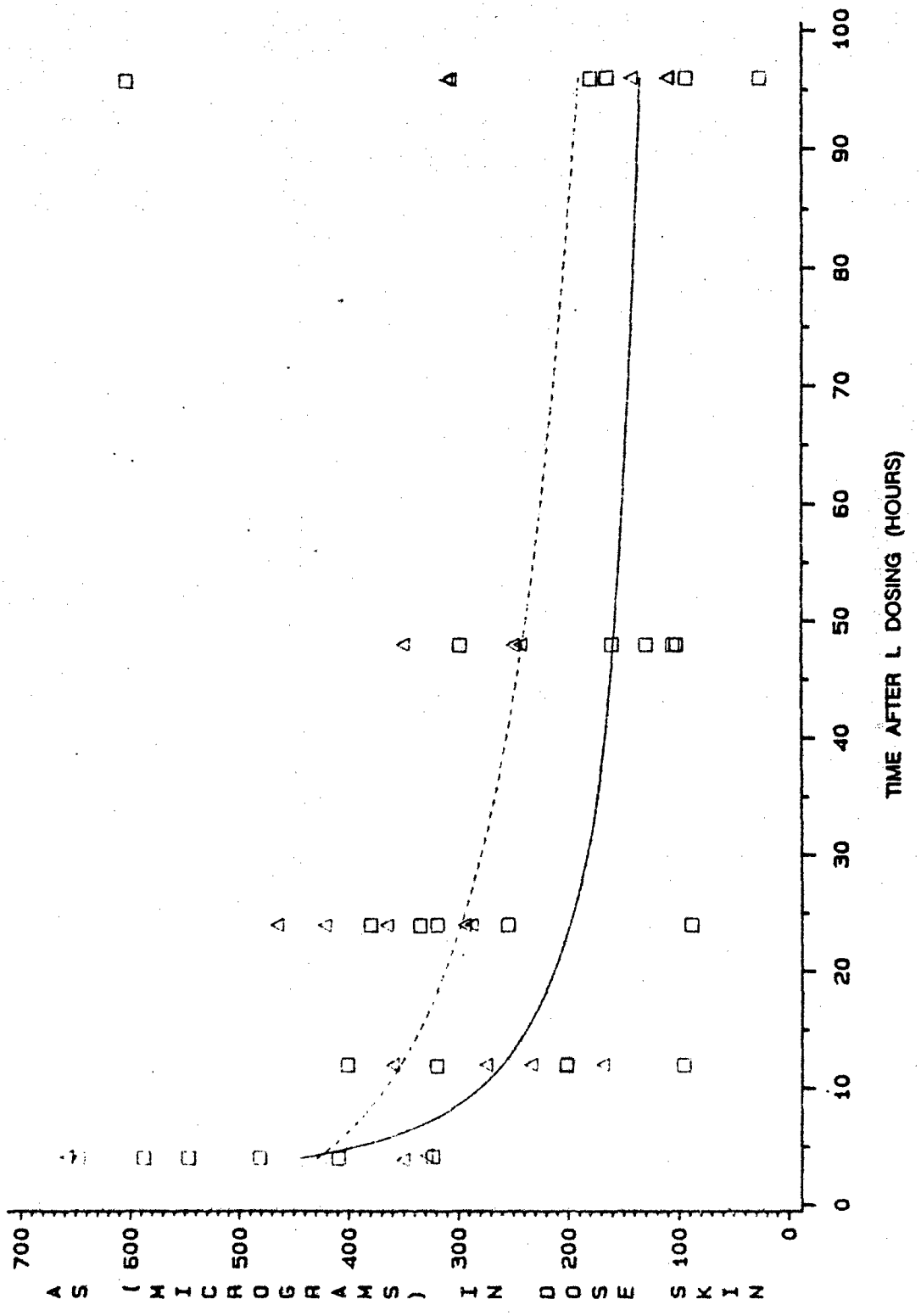


FIGURE 3.2.32 DOSE-SITE SKIN ARSENIC CONTENT (µg) AND REGRESSION CURVES FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

FIGURE 3.2.33 COMPARISON OF REGRESSION CURVES FOR WHOLE BLOOD ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

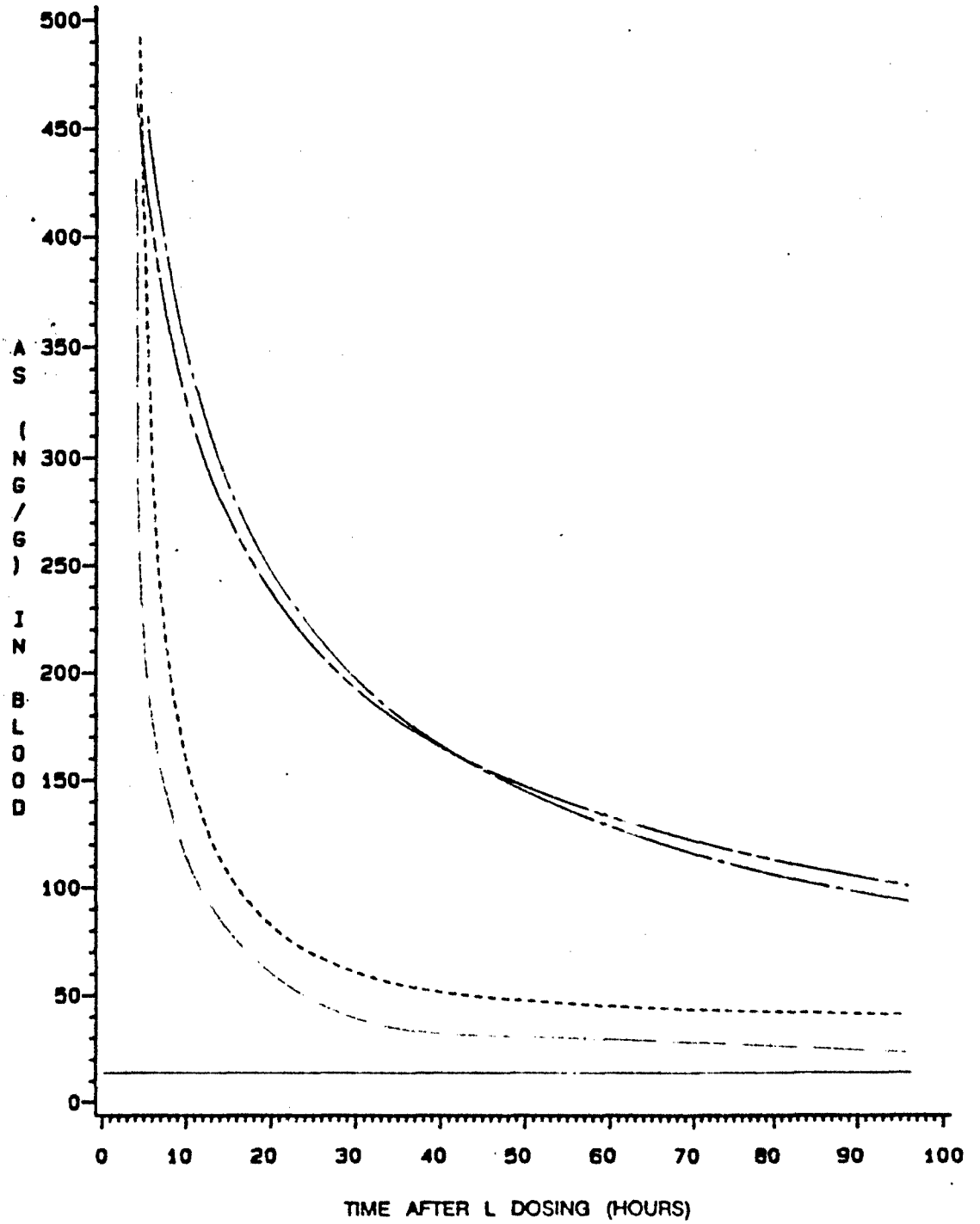


FIGURE 3.2.34 COMPARISON OF REGRESSION CURVES FOR BRAIN ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

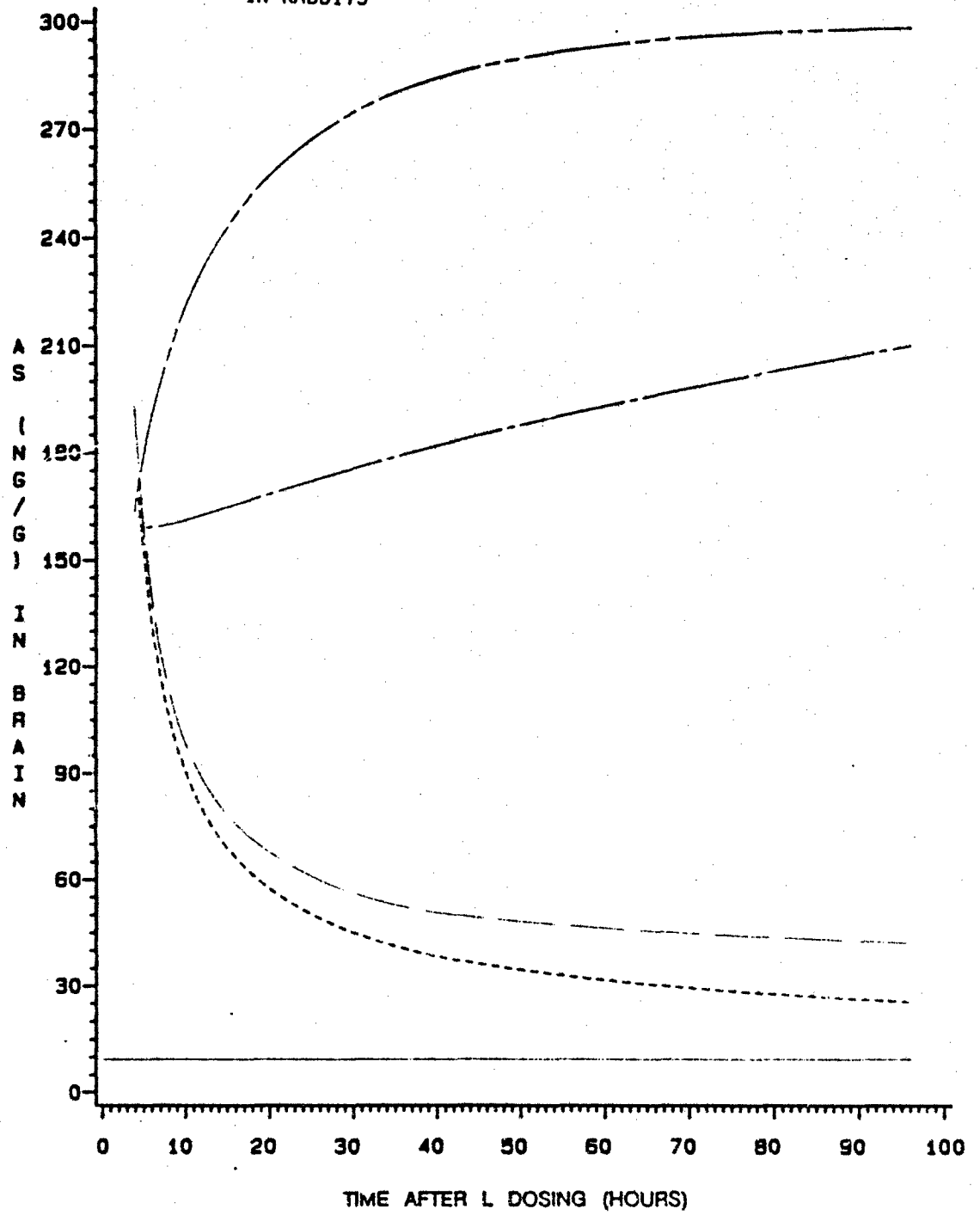


FIGURE 3.2.35 COMPARISON OF REGRESSION CURVES FOR SPINAL CORD ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

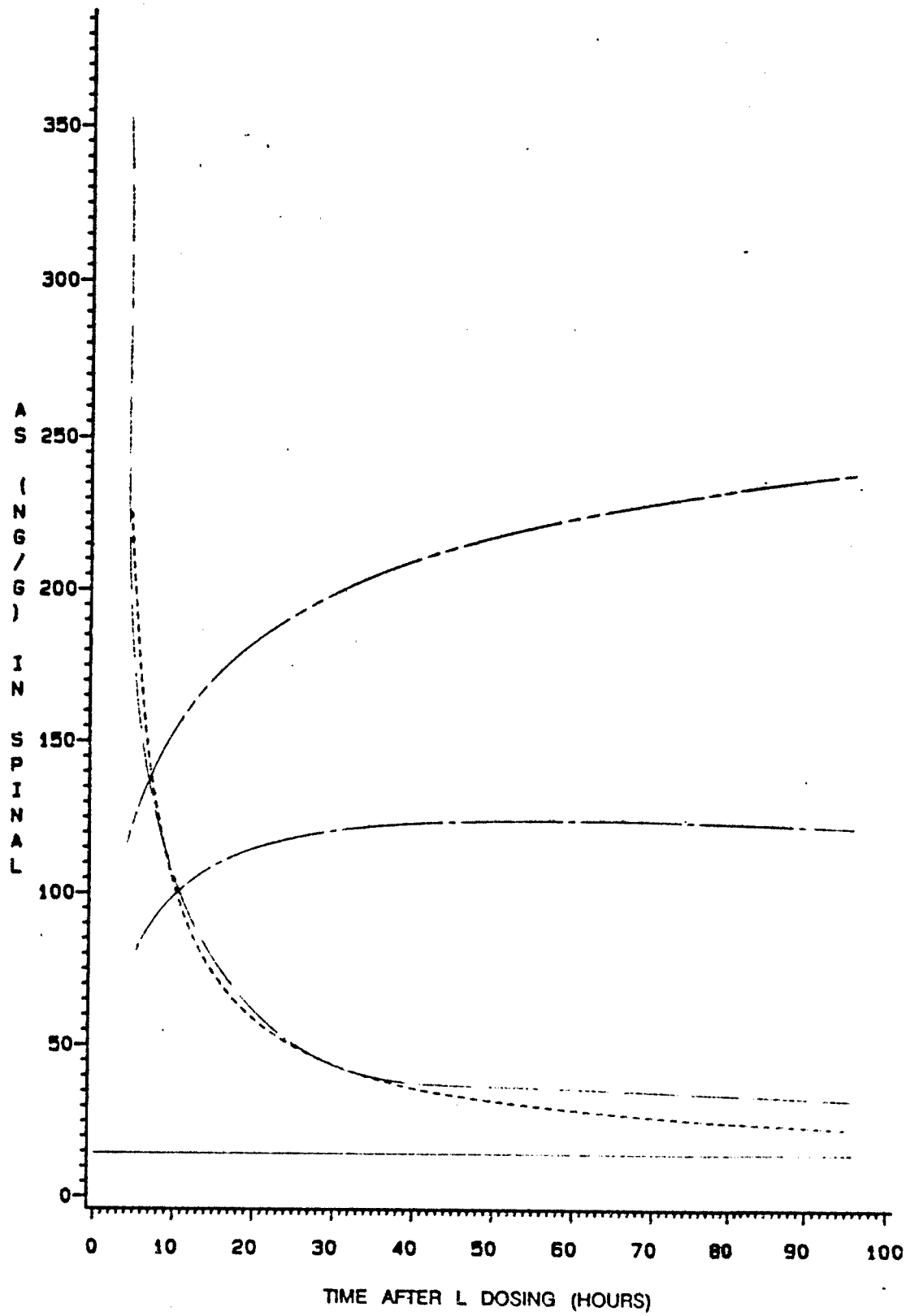


FIGURE 3.2.36 COMPARISON OF REGRESSION CURVES FOR RIGHT LUNG ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10}(2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

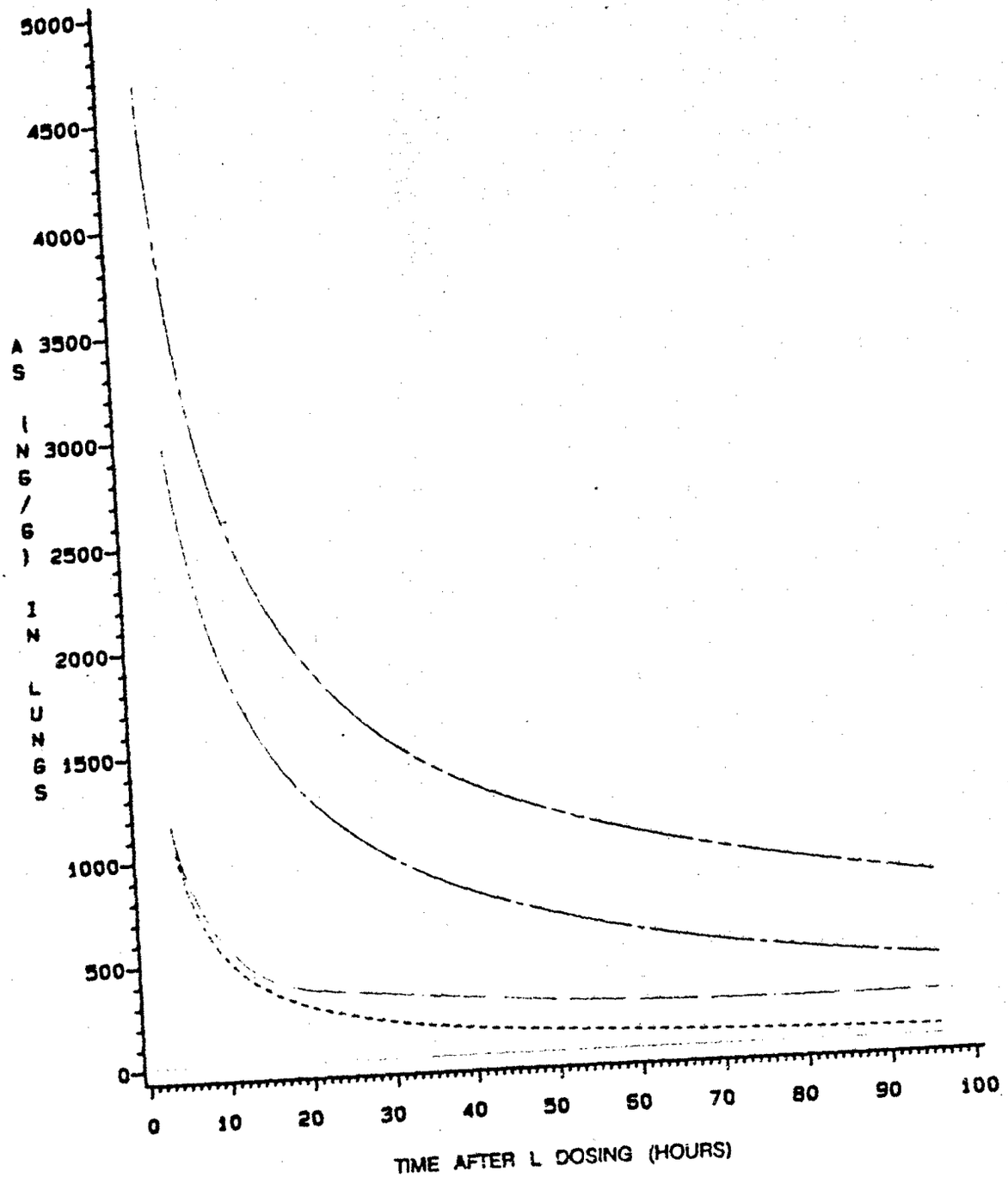


FIGURE 3.2.37 COMPARISON OF REGRESSION CURVES FOR LIVER ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

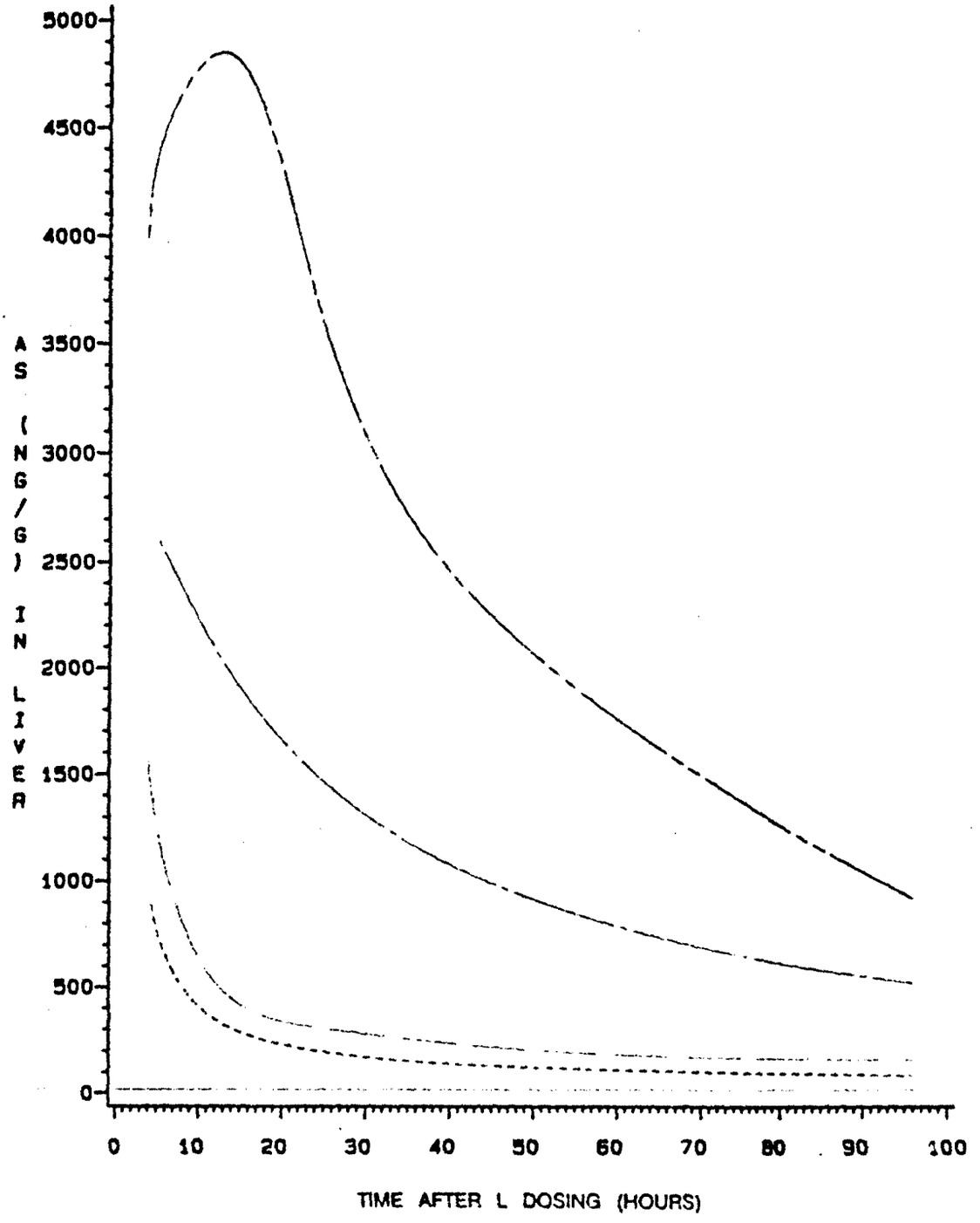


FIGURE 3.2.38 COMPARISON OF REGRESSION CURVES FOR KIDNEY ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

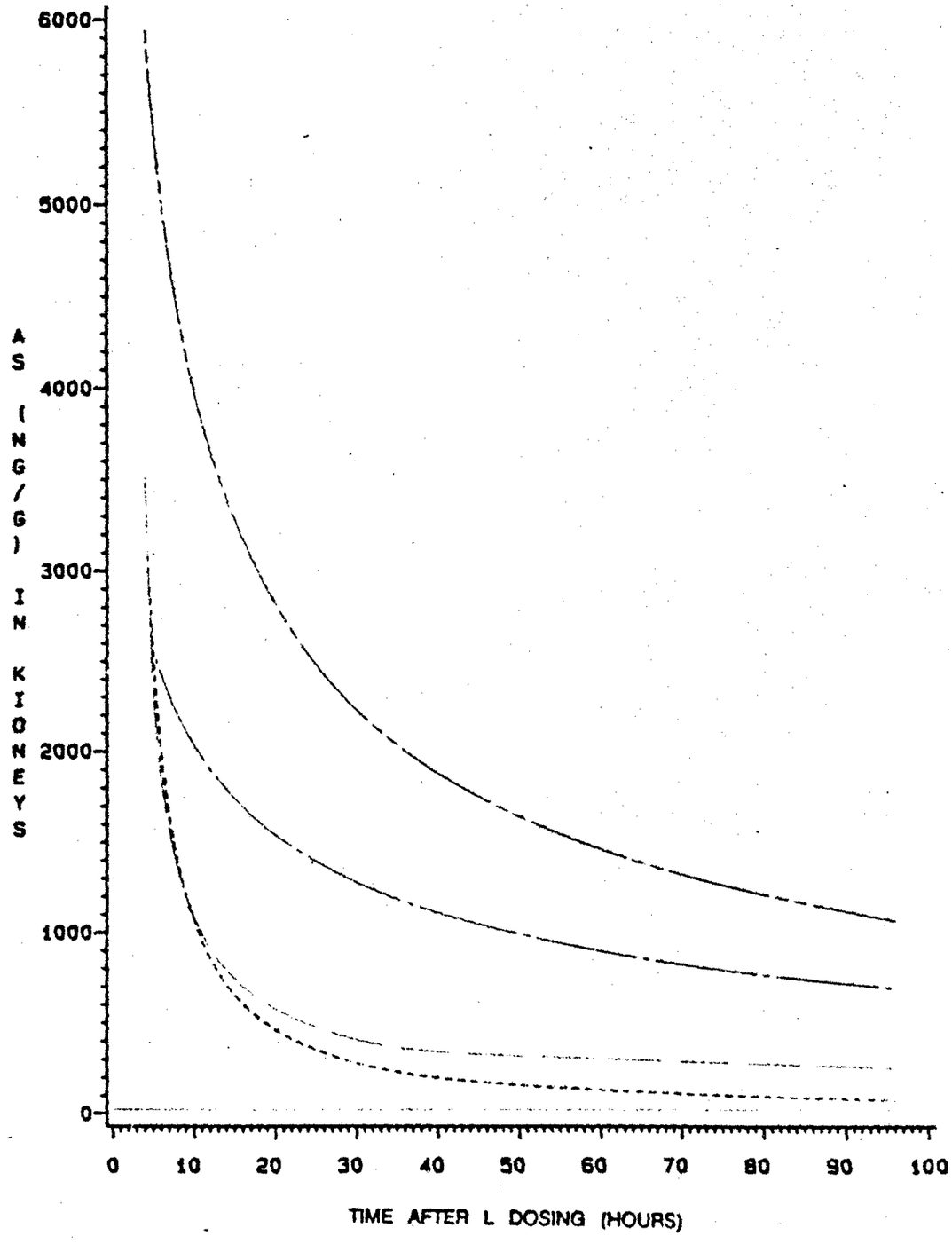


FIGURE 3.2.39 COMPARISON OF REGRESSION CURVES FOR RIGHT TESTIS ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

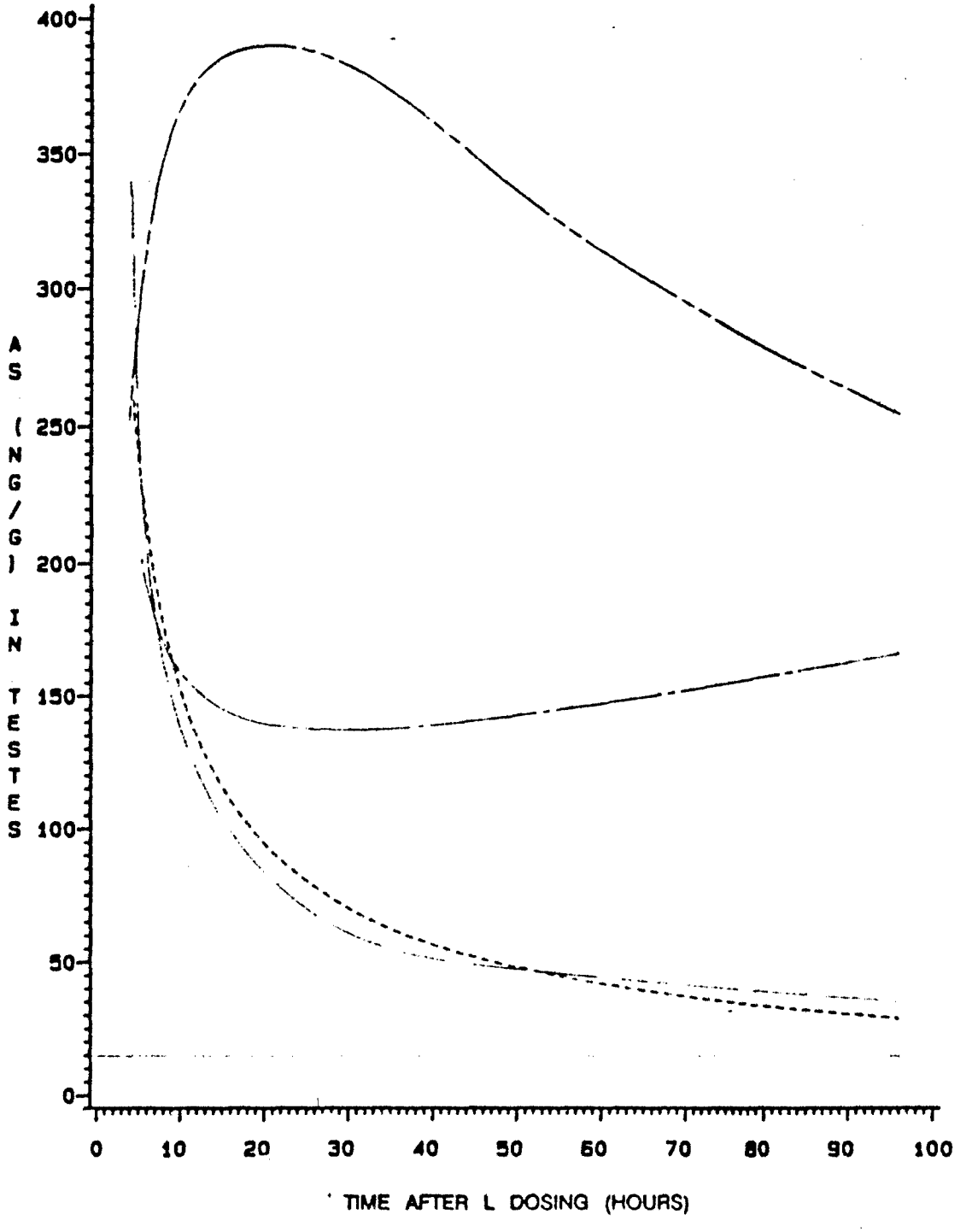


FIGURE 3.2.40 COMPARISON OF REGRESSION CURVES FOR ABDOMINAL FAT ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

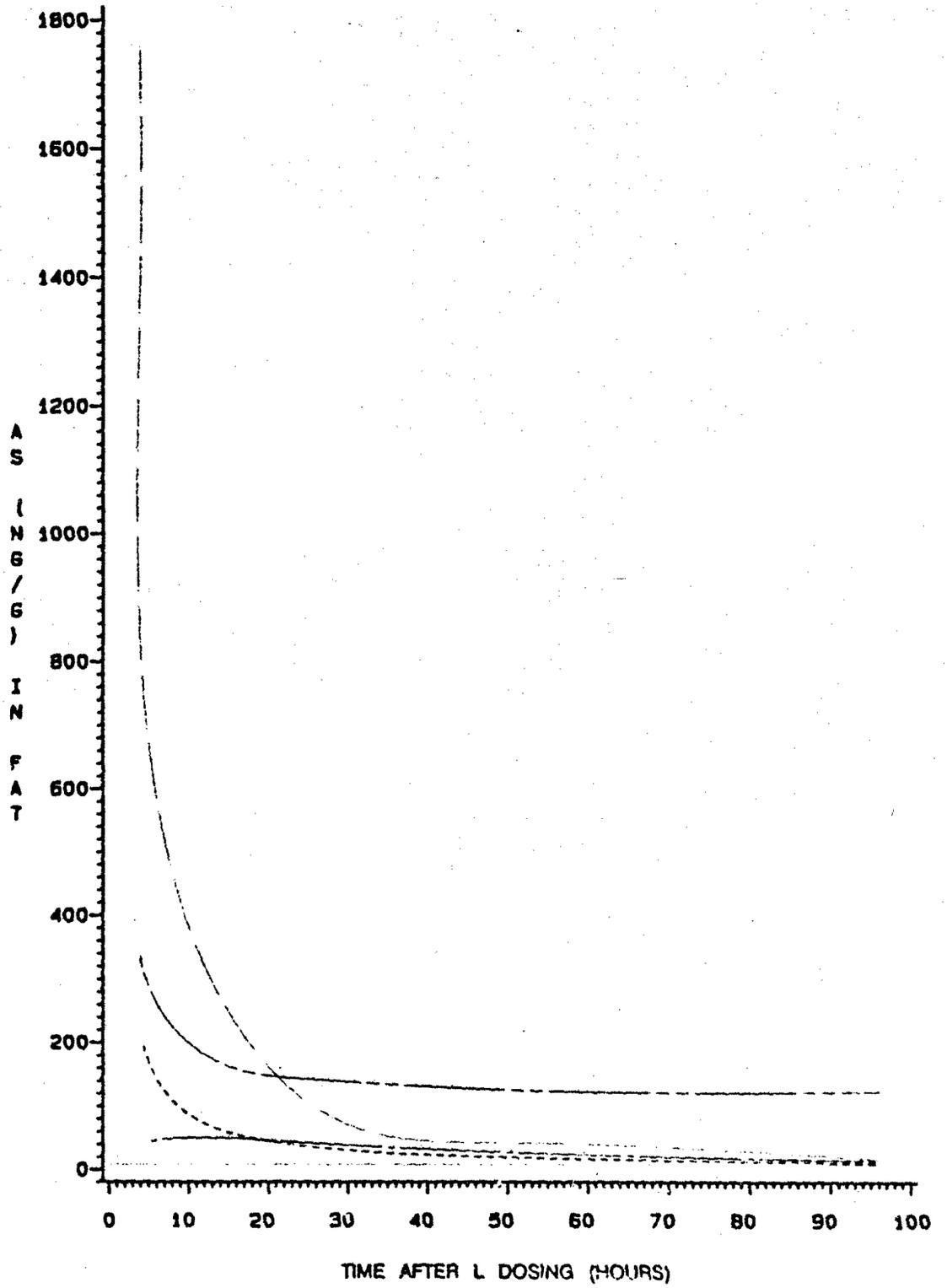


FIGURE 3.2.41 COMPARISON OF REGRESSION CURVES FOR DOSE-SITE SKIN ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

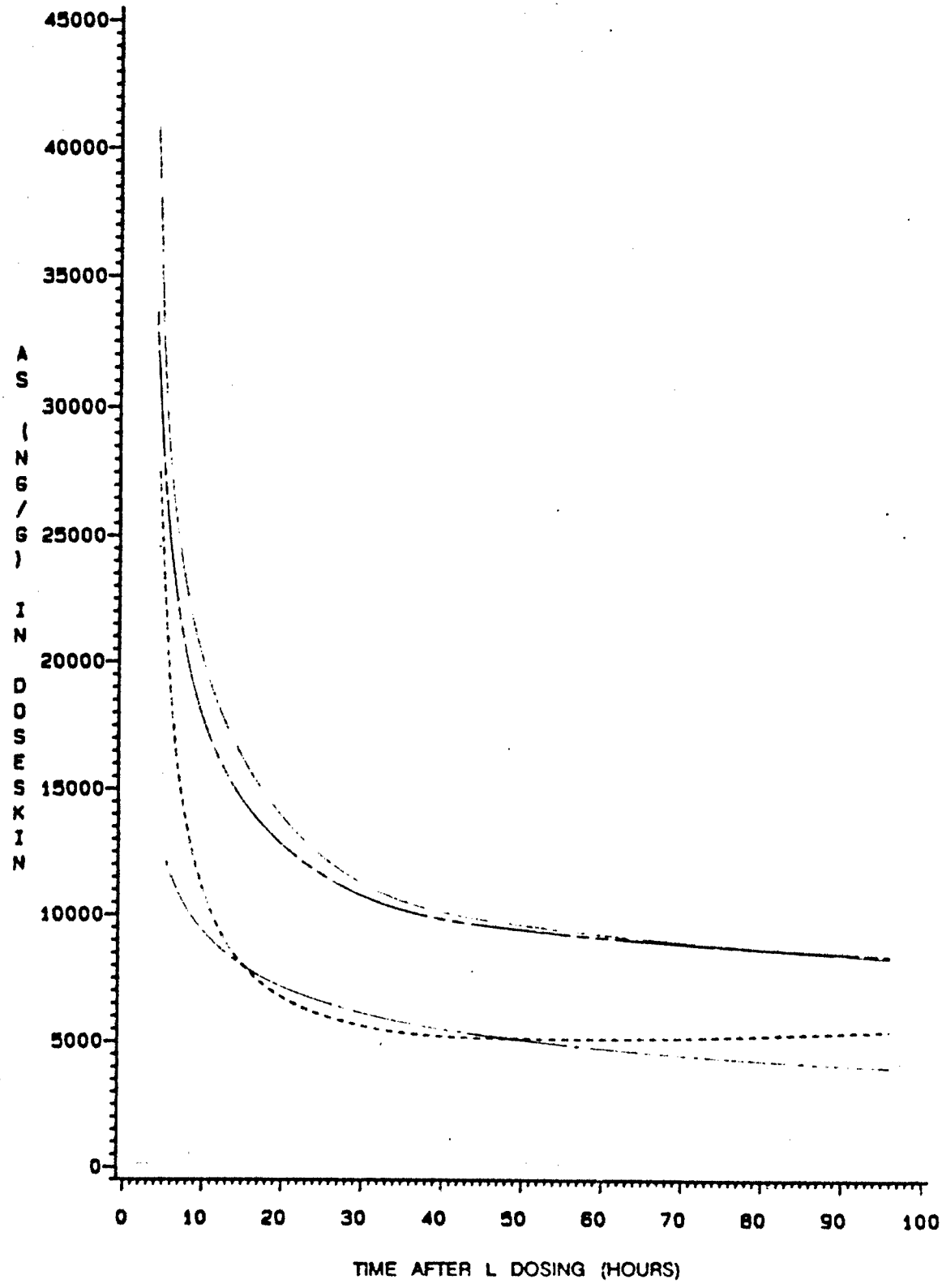


FIGURE 3.2.42 COMPARISON OF REGRESSION CURVES FOR NORMAL SKIN ARSENIC CONCENTRATIONS (ng/g) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

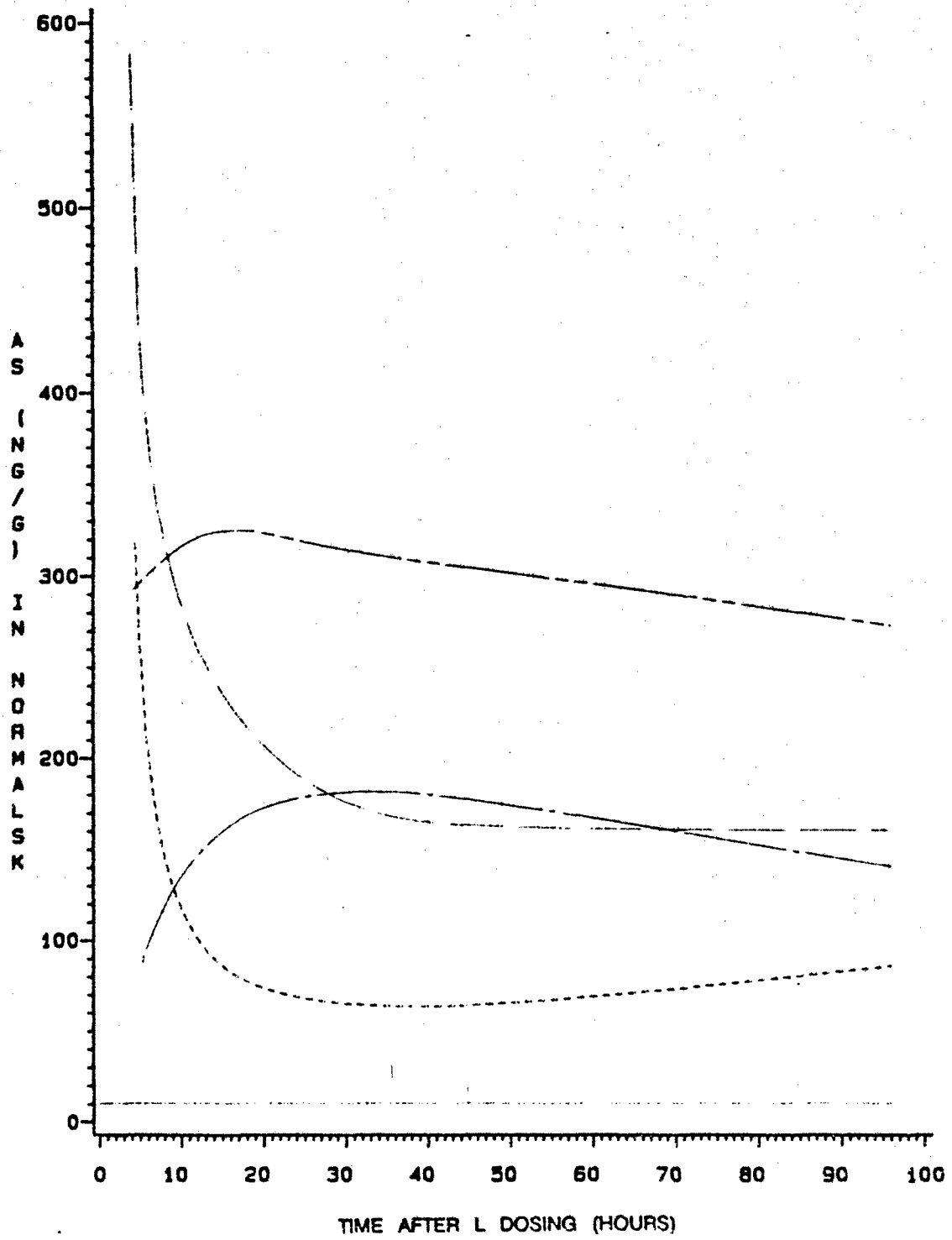


FIGURE 3.2.43 COMPARISON OF REGRESSION CURVES FOR WHOLE BRAIN ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

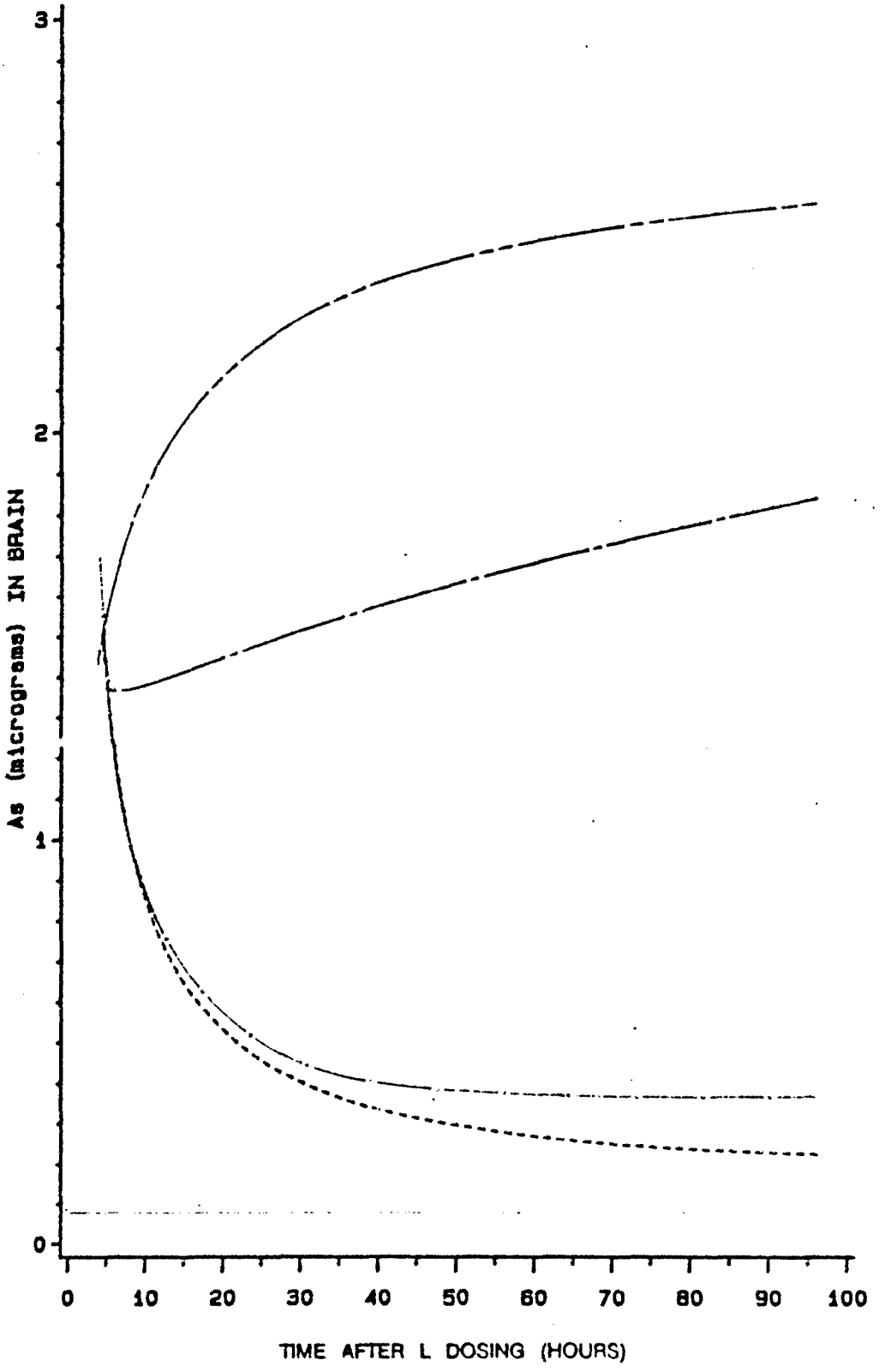


FIGURE 3.2.44 COMPARISON OF REGRESSION CURVES FOR WHOLE LUNGS ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

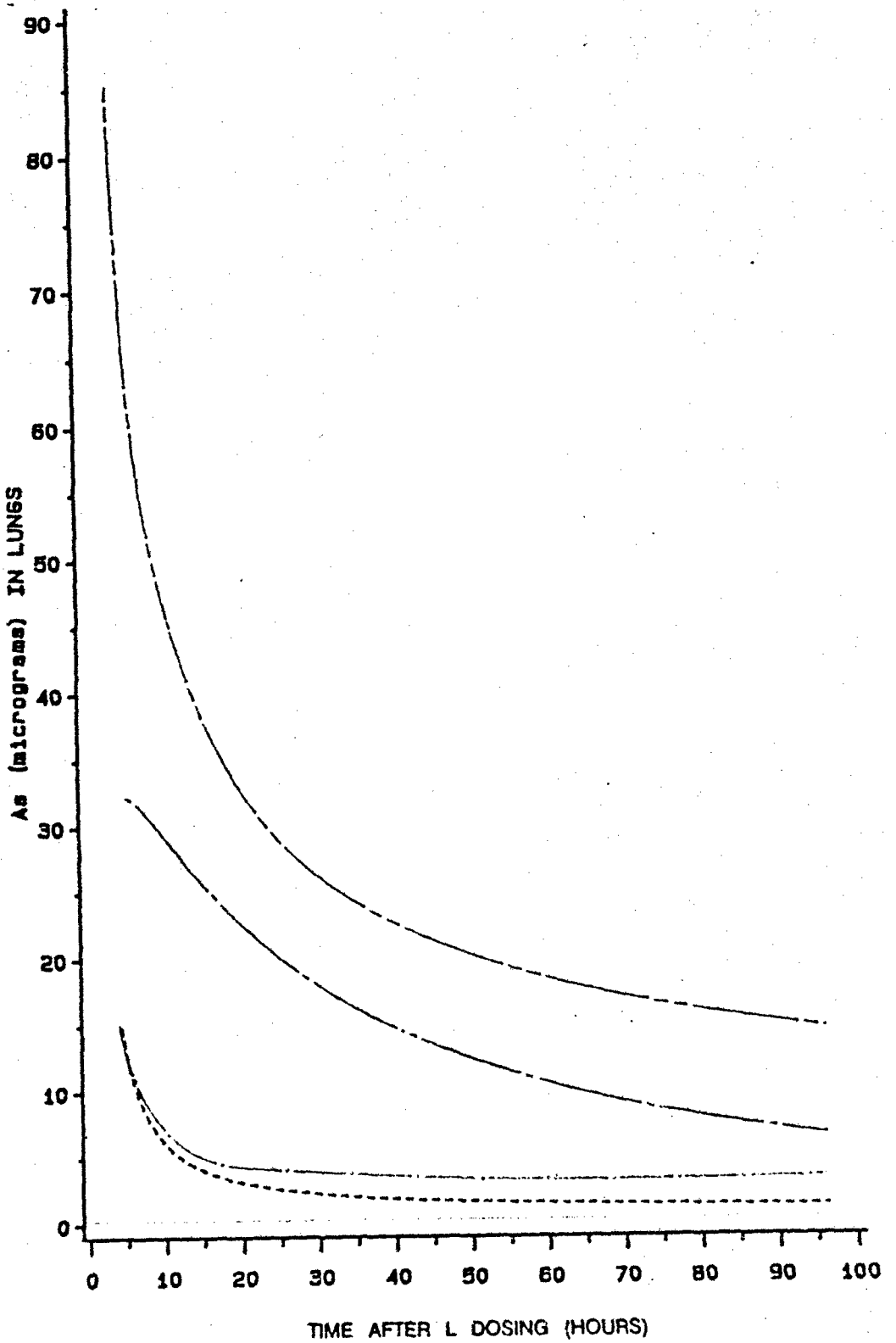


FIGURE 3.2.45 COMPARISON OF REGRESSION CURVES FOR WHOLE LIVER ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

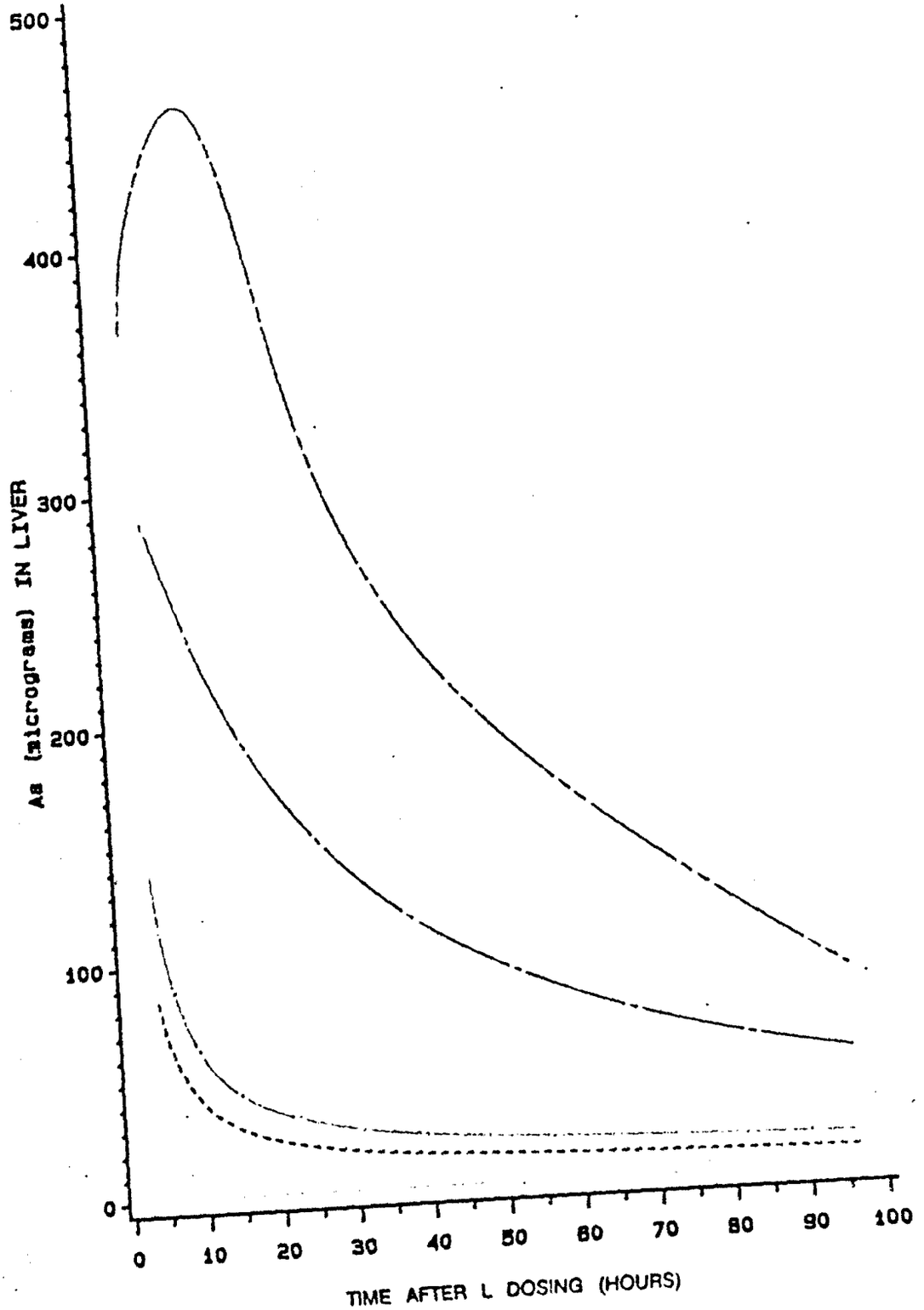


FIGURE 3.2.46 COMPARISON OF REGRESSION CURVES FOR WHOLE KIDNEYS ARSENIC CONTENT (µg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

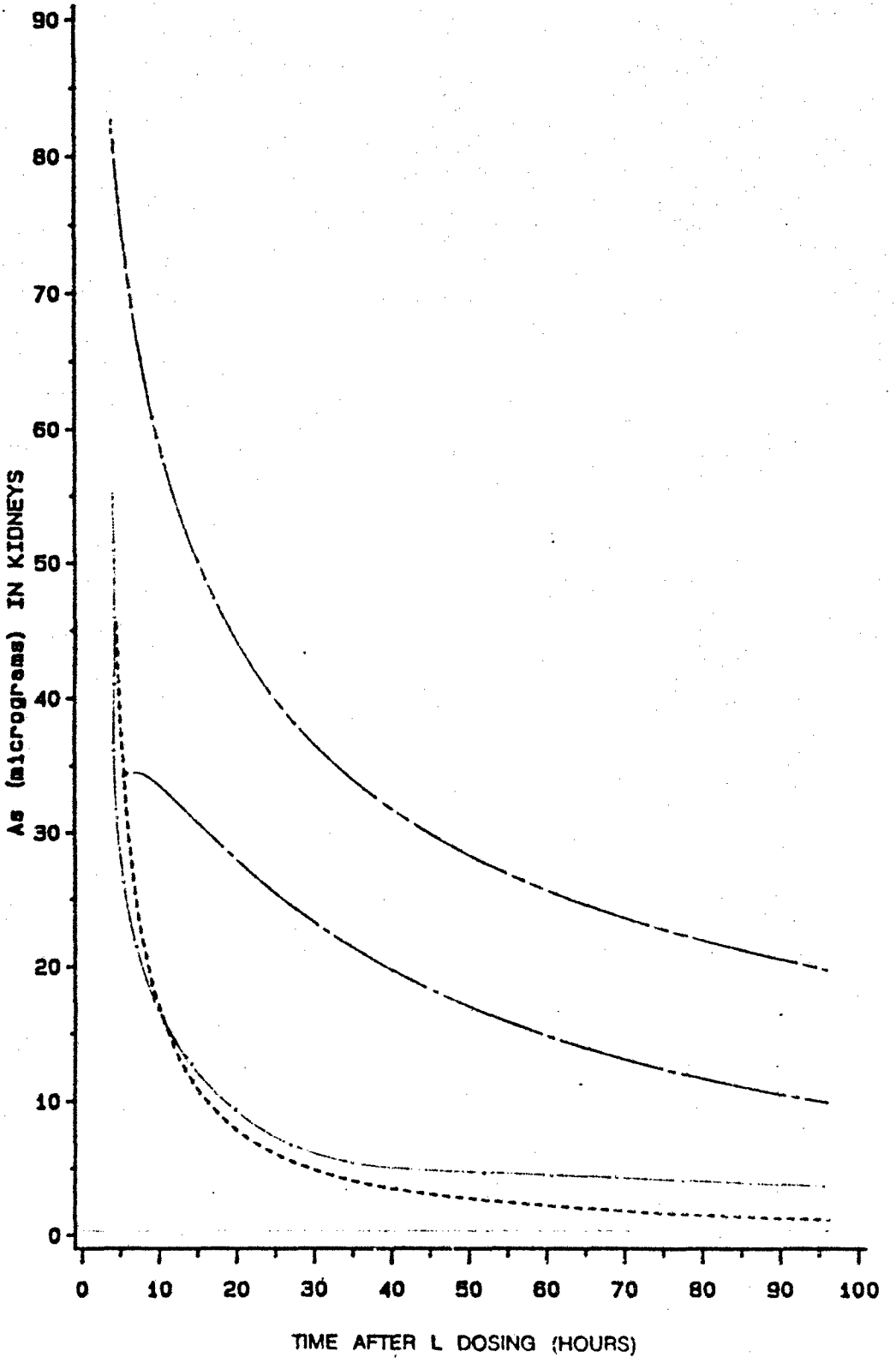


FIGURE 3.2.47 COMPARISON OF REGRESSION CURVES FOR WHOLE TESTES ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS

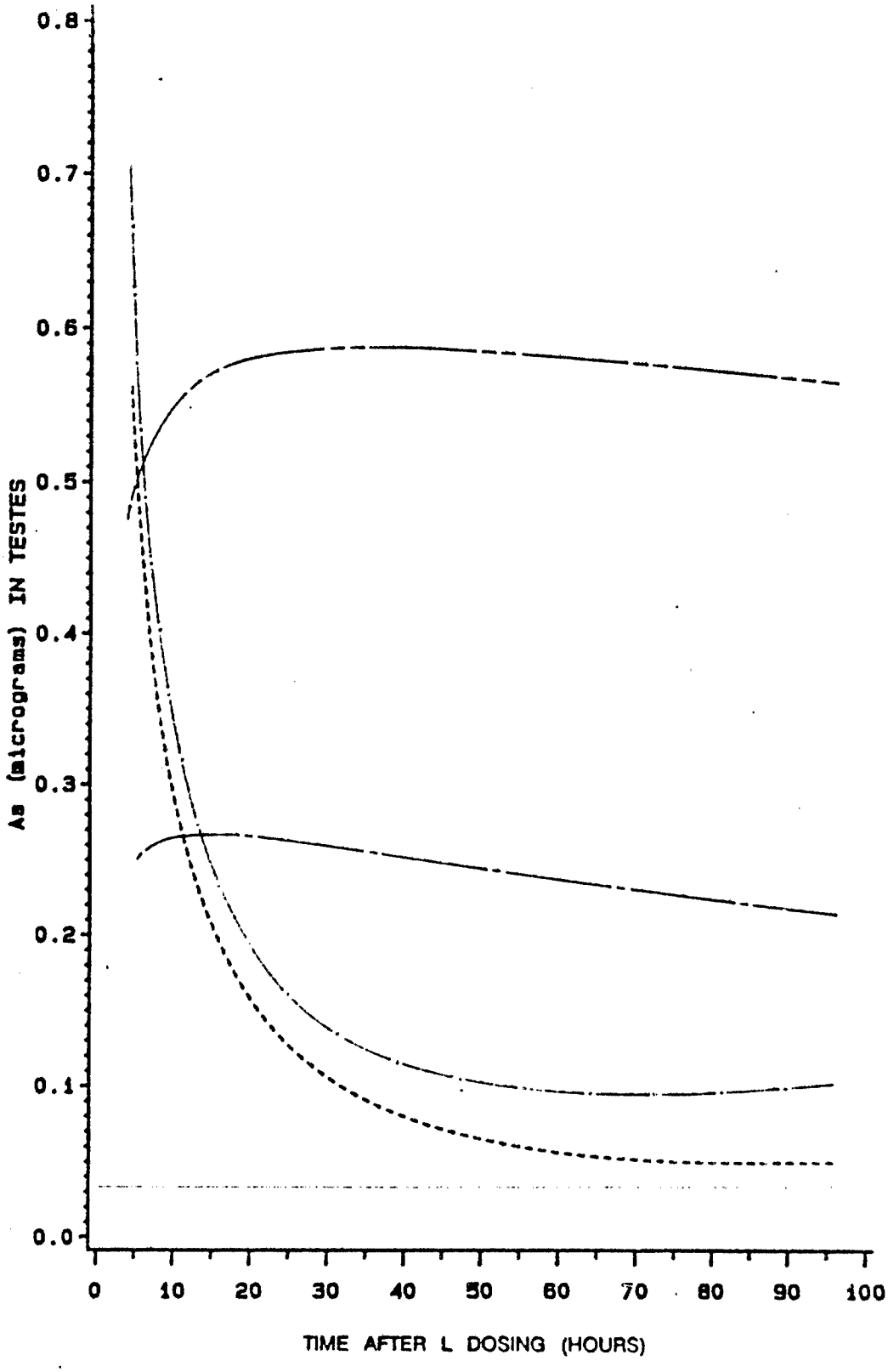
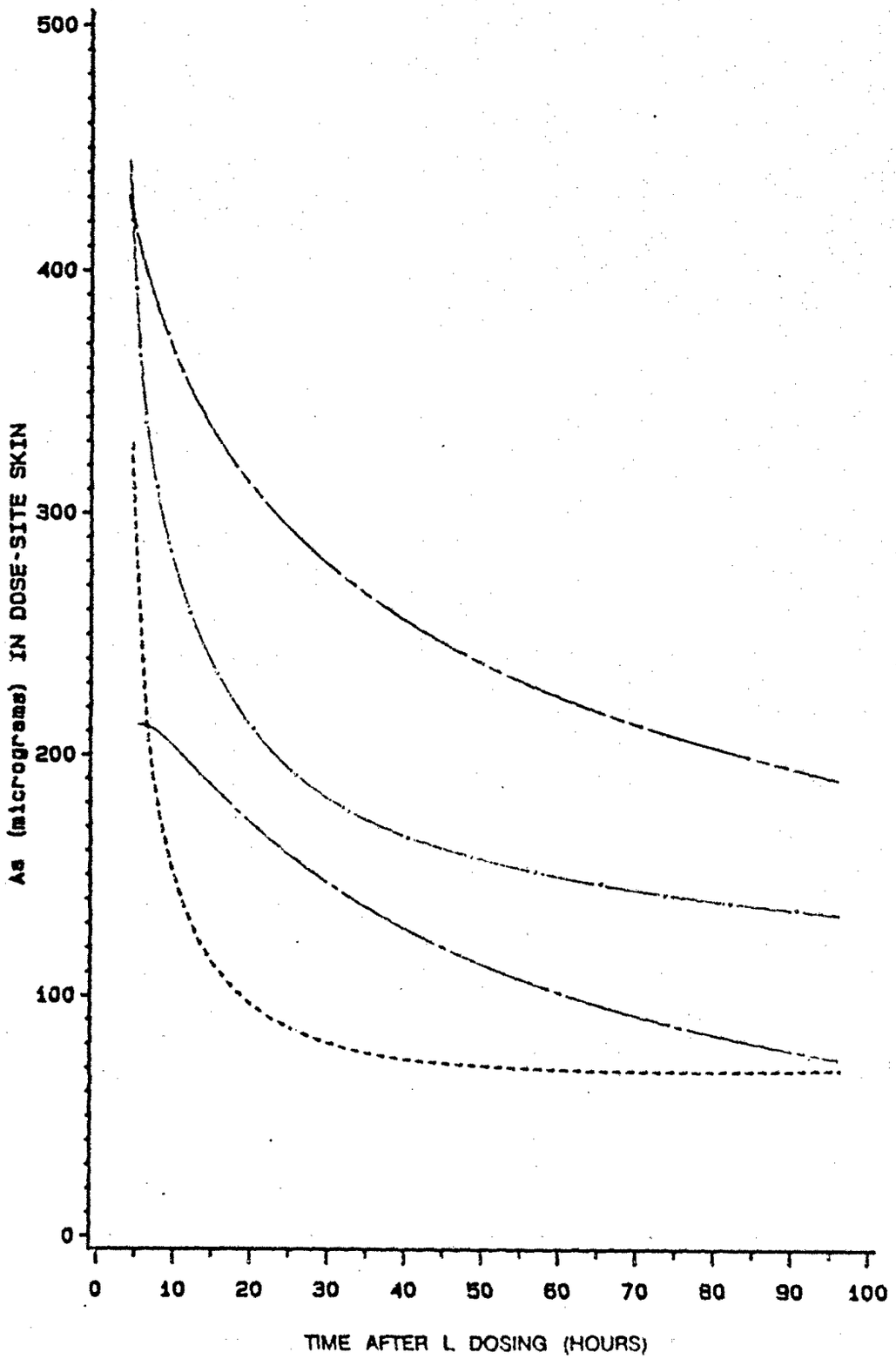


FIGURE 3.2.48 COMPARISON OF REGRESSION CURVES FOR DOSE-SITE SKIN ARSENIC CONTENT (μg) FOLLOWING SUBCUTANEOUS ADMINISTRATION OF L AT EITHER THE LD_{10} (2.4 mg/kg) OR THE LD_{40} (3.5 mg/kg) WITH AND WITHOUT BAL THERAPY IN RABBITS



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